Florida State Horticultural Society

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Front and Back Covers

Mangos in Florida and the World

Mango (*Mangifera indica* L.) originated in tropical south and southeast Asia about 4000 years ago. According to the United Nations, most production is found in the intertropical region, with six countries accounting for most of the world's production: India, China, Thailand, Indonesia, Mexico, and Pakistan. While most production is consumed domestically, a slightly different six countries supply most of the exports: Mexico, India, Thailand, Brazil, Pakistan, and Peru.

The early Florida State Horticultural Society Proceedings provide several reflections on the introduction of mango into Florida, plus early culture, and shipping and marketing by: A.A. Boggs (1903, vol. 16); P.H. Rolfs (1906, vol. 19); E.V. Blackman (1909, vol. 22); Mrs. P.H. Rolfs (1915, vol. 28); J.W. Barney (1924, vol. 37); Paul Heyman (1930, vol. 43); David Fairchild (1931, vol. 44; 1938, vol. 51); H.S. Wolf (1937, vol. 50); and many others.

The mango was first introduced to south Florida from Mexico in 1833, but likely did not survive. Additional attempts were made on the west coast with plants from Cuba and other countries over the next few decades, but also failed for various reasons including frosts. The first successful plantings were made in the Miami area in 1861 and 1868.

'Number 11' and 'Peach' mangos were recognized in the late 1800s. 'Number 11' arrived in Jamaica with French sailors prior to its arrival in Cuba and Florida. It is a fibrous yellow fruit now known as 'Turpentine'. In 1889, 'Mulgoba' and 'Alphonso' were the two surviving cultivars of the six introduced by the federal government (an agency that that later became the US Department of Agriculture).

'Turpentine' and 'Mulgoba' somehow crossbred in West Palm Beach, but the progeny were not recognized until in 1910, when a 'Mulgoba' seed in the back yard of Florence Haden of Coconut Grove produced what we now know as the 'Haden' mango. It was a beautiful fruit with an eye-stopping display of reds and yellows combined with an excellent flavor. It quickly thrived and dominated the mango industry of Florida. 'Haden' also proved to be a good parent, giving rise important export mango cultivars such as 'Kent' (Miami), 'Keitt' (Homestead), and 'Tommy Atkins' (Fort Lauderdale).

The turn of the 20th century was considered to be the beginning of a real mango industry in Florida. The 1918 catalog from Glen Saint Mary Nursery in Glen Saint Mary, FL. listed three sizes of mango trees, none priced higher than $1.00. Two former presidents of the Florida State Horticultural Society, G.L. Taber (1897–1904) and H. Harold Hume (1910–22), were the nursery's president and vice president, respectively.

Breeding programs around the world have worked to develop new mango cultivars. These efforts include field production criteria such as tree size, natural disease tolerance in blooms, as well as young and mature fruit, flavor, and productivity.

The 2017 Census of Agriculture reported approximately 2672 acres of commercial mangos grown in Florida—which means it is a small industry. Most are grown in Miami-Dade County, followed by Palm Beach, and Lee Counties.

To keep the industry viable in the face of competition from major exporting countries such as Mexico, Florida growers must become be creative, experimenting with new products and exploring new markets. Early cultivars such as 'Mallika', 'Nam Doc Mai', and 'Angie' can start the season in May. These cultivars generally have a small tree size, considerable disease tolerance, and excellent fruit quality. They also appeal to distinct ethnic groups.

The future of the Florida mango industry depends at least in part on the selection, testing, and adoption of new cultivars that can distinguish themselves in terms of eating quality and also in horticultural traits such as smaller trees, which are adapted to smaller plantings. Finding new cultivars and targeting new niches that increase profits are some of the challenges facing small-scale producers.

Farmers markets have become increasingly popular in south Florida, focusing more on locally-grown produce. Restaurants can easily adapt their menus to accommodate the seasonal availability of local produce compared to grocery stores and fast-food chains. Seasonal cooking has also become popular with the local food movement. Using the direct-from-farm sales model, local growers have been setting up their own distribution channels.

Front cover: An orchard of 'Lily' mango.

Back cover: Clockwise from the top left: 'Kent' mango orchard in Florida, 'Pickering' mango inflorescence, Dooryard mango in Florida, bowl of 'Rapoza' and 'Sunset' mangos

Photos by Noris Ledesma and George Fitzpatrick

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of the

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2020 PRESIDENTIAL ADDRESS

JEFFREY WILLIAMSON

President of the Florida State Horticultural Society

Fellow members and distinguished guests, welcome to the 133rd Annual Meeting of the Florida State Horticultural Society. I would like to begin by thanking the following volunteers who have helped make this meeting possible and keep the Society functioning. I'd also like to thank the six Sectional Vice Presidents who organized some exceptionally high-quality programs this year.

I will begin with the FSHS Officers and Board members–

- Gene McAvoy, Board Chair: thank you Gene for your capable leadership during these challenging times.
- Noris Ledesma, President Elect: Thank you Noris for your willingness to serve as future President and Board Chair.
- Secretary: Fernando Alferez.
- Treasurer: Kevin Athearn.
- Editor: Mary Lamberts.
- Program Coordinator: Cami McAvoy.
- Student Awards Chairperson: Michelle Danyluk.
- Meeting Poster Coordinator: Taylor Clem.
- Newsletter Editor: Alicia Lamborn.
- Board Members-at-Large: Adrian Hunsburger and Tom Stopyra.
- Marketing Coordinator and Webmaster: Steve Rogers and Mark Ritenour.

I'd also like to thank—

- Gene McAvoy for organizing this year's IST.
- Our friends from ASHS.
- Patron members: Theodore Winsberg, Anne Plotto, and Ed Etxeberria.

I'd like to thank the UF/IFAS administration for their support. In particular, I'd like to thank—

- Dean for Extension: Nick Place.
- Dean for Research: Robert Gilbert.
- Associate Dean for Extension: Saqib Mukhtar.
- Jackie Burns, acting chair, Horticultural Sciences Dept.
- Dean Kopsell, chair, Environmental Horticulture Dept.

A special shout out to the IFAS IT team helping us through the maze in our first attempt at a virtual annual meeting: Dewayne Hyatt, Joe Gasper, and Kevin Hill.

A society of this complexity, with this many moving parts, requires a great deal of additional volunteer support in the form of committee assignments. As President, I appointed 11 committees–some were quite large. Many of you stepped up to serve on these committees which are so essential to making our meeting run efficiently. You are the unsung heroes of our society. I want to express my sincere appreciation to those of you who volunteered your time and energy to participate in that capacity.

These are very uncertain times we are living in. We are experiencing the worst pandemic since the 1918 Pandemic, which is sometimes referred to as the "Spanish Flu". The 1918 Pandemic is estimated to have infected about one-third of the world's population. The death toll was tremendous, estimates range from 50 to 100 million worldwide, with 675,000 deaths in the U.S. It was compounded by the fact that World War I was ongoing. Little could be done to medically treat the disease at the time. There were no effective vaccines, or antibiotics to treat secondary infections. Control efforts were limited to non-pharmaceutical interventions such as isolation, quarantine, use of disinfectants, good personal hygiene, and limitations on public gatherings. Masks were commonly worn and required in many cases. Does this sound all too familiar to you? It probably does. Florida did not escape this tragic event. At one point one-third of the University of Florida students were infected with the virus. For the past six months, our lives have been turned upside down. This year, your Board struggled with how to address the situation with respect to our annual meeting. Since its inception in 1888, the Florida State Horticultural Society has met annually 132 times, never missing a beat, to discuss the science, business, and state of horticulture in Florida. Our society met unabated during the difficult period impacted by the 1918 Pandemic. I will be the first to say that I will be glad to see you all in person again when things return to normal. However, this situation we find ourselves in today did get me thinking about our Society during that difficult period in the last nineteen teens. I wondered about these seemingly fearless people who met despite the pandemic. Did they understand the

risks they were taking? I believe they probably did. After reading the President's address, delivered by Harold Hume (a former UF president), it became apparent to me that they were consumed by what they perceived to be a far greater threat than the relatively new threat of the pandemic; rather the threat of war which had been raging in Europe for several years. President Hume's address did not mention the 1918 Flu but focused entirely on the war effort and what those in attendance should do to support their country during that difficult period. To me, the fact that they met at all under such difficult circumstances demonstrates the importance of the FSHS to their daily lives. Aside from President Hume, a few other notable people in attendance at the 1918 meeting included W.J. Krome (railroad builder and tropical fruit pioneer), Wilson Popenoe (Agricultural Explorer and the first director of the Panamerican Agricultural School, Zamorano, Honduras), and P.H. Rolfs (UF Dean and Director of the College of Agriculture for whom Rolfs Hall on the UF campus is named). An influential group of individuals to say the least.

Fast forward about a century and we find ourselves striving to carry on the rich traditions of this society while at the same time not putting our membership at risk of contracting the coronavirus. With the rise in coronavirus cases throughout the summer, the possibility of an on-site meeting was out of the question. Thankfully, today's technology allows us to meet virtually from the safety of our homes and offices. I want to thank Gene McAvoy for reaching out to our IFAS IT specialists to help us implement the TEAMS platform for this virtual meeting. I also want to thank Tracy Shawn from ASHS for renegotiating our contract with the conference hotel to move the meeting date to 2022 without any penalty. I also want to thank Mark Ritenour for doing a yeoman's job of putting the collection of past FSHS proceedings online, so that we have the history of Florida horticulture at our fingertips. I encourage you all to look at some of the older proceedings as they make for very interesting reading. As I mentioned before, I look forward to when we can once again meet in person, but for now, I hope you will enjoy and benefit from this year's first virtual FSHS annual meeting.

Before I conclude my remarks, I would like to emphasize that our society operates solely on a volunteer basis. Thus, our society is only as strong as our membership. I encourage each and every one of you to step up and volunteer some of your time and expertise to keep our society strong and functioning efficiently. There are many standing committees as well as specially appointed committees that need your help. Educating and training students is a large part of what we do and our annual meetings provide the perfect environment to give students the added experience of presenting their work to their peers before they participate in a national or international meeting. Please continue to support and encourage your students to participate in our annual meetings. They represent our future. Authors, please submit your manuscripts in a timely manner. This alone will be a tremendous help for maintaining the high quality of our proceedings which are among the very best of any state horticultural society or similar organization in the country. Remember that our proceedings not only provide current and relevant research results for Floridians, but they also serve as a comprehensive source for the history of Florida horticulture.

Last, let me say that it has been my distinct pleasure to serve as your president and I hope you find this year's program informative, productive, and enjoyable. Thank you.

2020 Awards of the Society

2020 MERITORIOUS SERVICE AWARDS

For significant contributions to Florida horticulture

Presidential Gold Medal Award

For having contributed the most to Florida horticulture through work published in the Proceedings of FSHS over the preceding five-year period

Shawn Steed

Ornamental, Landscape and Gardening Section

Outstanding Commercial Horticulturist Award

Presented to the person who has made significant and historical contributions to Florida's commercial horticulture industry and to FSHS's Krome Memorial Institute

Noris Ledesma

Krome Memorial Institute

President's Industry Award

The award is presented for the best paper given at the FSHS annual meeting during the previous year by an industry author.

Nicholas A. Larsen

Growing Bananas in the Everglades Agricultural Area
Proc Fla. State Hort. Soc. 132:32–36. 2019.

2020 Patron Members

Members who annually pledge additional support for the student programs of the Society

Ed Etxeberria

Anne Plotto

Theodore Winsberg

Friends of the Florida State Horticultural Society Award

To recognize individuals for their longtime commitment and support of the Florida State Horticulture Society

(not awarded)

Florida Tomato Research Award

This award shall be given to the individual or group who have done work with the most potential to further the fresh market tomato industry in Florida through advances reported in any single publication during the two (2) previous calendar years

(not awarded)

SECTIONAL BEST PAPER AWARDS

From the Proceedings of the Florida State Horticultural Society Volume 132

Awarded to the senior author of the best and most meritorious paper as printed in the previous year's Proceedings in each of the six sections

Agroecology and Natural Resources Section

Lyn A. Gettys

Herbicide Efficacy on the Small Floating Weeds Redroot Floater and Feathered Mosquitofern
Proc. Fla. State Hort. Soc. 132:241–244. 2019.

Authors: Lyn A. Gettys, Kyle L. Thayer, Joseph W. Sigmon, Ian J. Markovich, and Mohsen Tootoonchi

Fort Lauderdale Research and Education Center, University of Florida/IFAS, Fort Lauderdale, FL

Citrus Section

Pictured: **Rayane Barcelos Bisi** (left) and **Ute Albrecht** (co-author, right)

Seed and Seedling Nursery Characteristics for 10 USDA Citrus Rootstocks
Proc. Fla. State Hort. Soc. 132:88–92. 2019

Authors: Rayane Barcelos Bisi[1], Ute Albrecht[1], and Kim D. Bowman[2]

[1]Southwest Florida Research and Education Center, University of Florida/IFAS, Immokalee, FL [2]US Horticultural Research Laboratory, USDA, ARS, Fort Pierce, FL

SECTIONAL BEST PAPER AWARDS (continued)

Handling & Processing Section

Anna Marín

Optimizing Essential Oil Applications to Prevent Postharvest Decay in Strawberries
Proc. Fla. State Hort. Soc. 132:185–188. 2019

Authors: Anna Marín[1], Xiuxiu Sun[1], Marcela Miranda[1], Chris Ference[1], Elizabeth Baldwin[1], Jinhe Bai[1], Mark Ritenour[2], and Anne Plotto[1]

[1]USDA-ARS, US Horticultural Research Laboratory, Fort Pierce, FL;
[2]Indian River Research and Education Center, University of Florida/IFAS, Fort Pierce, FL

Krome Memorial Institute

Jeffrey G. Williamson

Use of Sparkleberry as a Potential Rootstock in Commercial Blueberry Production
Proc. Fla. State Hort. Soc. 132:40–42. 2019.

Authors: Jeffrey Williamson and Rebecca Darnell
Horticultural Sciences Department, University of Florida/IFAS Gainesville, FL

Ornamental, Garden & Landscape Section

Thomas H. Yeager

Rain Impacts Container Plant Irrigation
Proc. Fla. State Hort. Soc. 132:203–204. 2019.

Author: Thomas H. Yeager
Environmental Horticulture Department, University of Florida/IFAS Gainesville, FL

Vegetable Section

Wendy Mussoline

Improved Productivity and Economic Advantages of Advanced Cabbage Production on Plasticulture
Proc. Fla. State Hort. Soc. 132:108–110. 2019.

Authors: Wendy Mussoline[1], Bonnie Wells[2], Gary England[3], and Lincoln Zotarelli[4]

[1]University of Florida/IFAS Extension Flagler/Putnam County, Bunnell, FL; [2]University of Florida /IFAS Extension St. Johns County, St. Augustine, FL; [3]University of Florida /IFAS Hasting Agriculture Extension Center, Hastings, FL; [4]Horticultural Sciences Department, University of Florida /IFAS, Gainesville, FL

BEST STUDENT ORAL PRESENTATION AWARDS

First—**Jose Hernandez-Monterroza** and Shinsuke Agehara
Gulf Coast Research and Education Center, University of Florida/IFAS, Wimauma, FL

Title: **Post-planting Root Growth Dynamics and Morphology of Bare-root and Plug Strawberry Transplants and Their Impacts on Field Performance**

Second—**Samuel Kwakye**, Davie Kadyampakeni, Tripti Vashisth
Citrus Research and Education Center, University of Florida/IFAS, Lake Alfred, FL

Title: **Evaluation of Varying Rates of Manganese on HLB-affected Trees (Valencia) in Florida**

Third —**Susmita Gaire**, Fernando Alferez, Ute Albrecht
Southwest Florida Research and Education Center, University of Florida/IFAS, Immokalee, FL

Title: **Canopy Growth and Physiological Assessment of 'Valencia' Orange Trees (*Citrus sinensis*) With and Without Individual Protective Covers**

STUDENT BEST WRITTEN PAPER AWARDS

These awards are given to encourage student participation in the published FSHS Proceedings, and are given to the students with the best papers submitted for publication in the Proceedings.

Best Written Papers from the 2018 Proceedings

First—**Stephen Deschamps** *(not pictured)* and Shinsuke Agehara
Gulf Coast Research and Education Center, University of Florida/IFAS, Wimauma, FL

Title: **Optimization of Microenvironments By Metalized-striped Plastic Mulch Improves Earliness and Total Yield of Strawberry**

Proc. Fla. State Hort. Soc. 131:164–170. 2018

Second—**Yanlin Wang** *(pictured, right)*, Yue, Y., McKenzie De New, and Bala Rathinasabapathi
Horticultural Sciences Dept., University of Florida/IFAS, Gainesville, FL

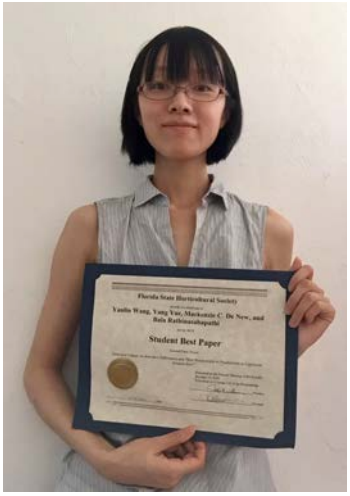
Title: **Stem and Canopy Architecture Differences and Their Relationship to Productivity in Capsicum annuum Lines**

Proc. Fla. State Hort. Soc. 131:121–125. 2018

Third—**Prabhiot Kaur** *(not pictured)*, Daniel Stanton, Jude Grosser, Manjul Dutt.
Citrus Research and Education Center, University of Florida/IFAS, Lake Alfred, FL

Title: **Genetic Modification of Citrus Utilizing Protoplasts Derived from Either Cell Suspension Cultures or Embryogenic Callus**

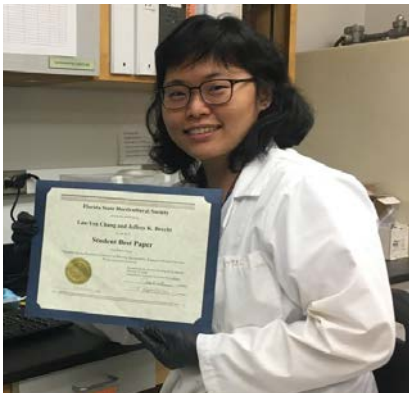
Proc. Fla. State Hort. Soc. 131:65–69. 2018.



Yanlin Wang

Best Written Papers (from the 2019 Proceedings)

First



Lan-Yen Chang

Variation Among Strawberry Cultivars in Bruising Susceptibility Related to Wound Ethylene Production and Sensitivity

Proc. Fla. State Hort. Soc. 132:196–200. 2019.

Authors: Lan-Yen Chang and Jeff Brecht
Horticultural Sciences Department, University of Florida/IFAS, Gainesville, FL

Second

Rayane Barcelos Bisi

Seed and Seedling Nursery Characteristics for 10 New USDA Citrus Rootstocks

Proc. Fla. State Hort. Soc. 132:88–92. 2019

Authors: Rayane Barcelos Bisi[1,2], Ute Albrecht[1], and Kim D. Bowman[2]
[1]Southwest Florida Research and Education Center, University of Florida /IFAS, Immokalee, FL;
[2]US Horticultural Research Laboratory, USDA, ARS, Fort Pierce, FL

Best Written Papers from the 2019 Proceedings (continued)

Third

John Santiago

Influence of Rootstock and Soil Environment on Citrus Rhizosphere Composition
Proc. Fla. State Hort. Soc. 132:54–61. 2019

Authors: **John Santiago**, Ute Albrecht, and Sarah L Strauss

Southwest Florida Research and Education Center, University of Florida /IFAS, Immokalee, FL

Sponsorship for Best Written Papers

Partial sponsorship by Mary Lamberts in honor of her father, J.J. Lamberts, who was a Professor of English at Arizona State University, specializing in contemporary English usage.

Student Scholarship Recipients

None awarded for 2020

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Albrigo, L. Gene	2012
Anderson, J.B.	1922
Anderson, Shirley F.	2002
Beckenbach, J.R.	1967
Berckmens, P.J.	1893
Berger, E.W.	1940
Berry, Robert E.	1987
Blackmon, G.H.	1964
Bosanquet, L.P.	1924
Brecht, Jeffrey K.	2014
Brown, Arthur C.	1952
Bryan, Herbert H.	2003
Bullock, Robert C.	2008
Burgis, Donald S.	1980
Calvert, David V.	1997
Camp, A.F.	1956
Campbell, Beverly	2010
Campbell, C.W.	1988
Cantliffe, Daniel J.	2006
Carlton, R.A.	1962
Castle, William S.	2011
Chase, J.C.	1939
Chase, S.O.	1939
Childers, N.F.	1993
Clayton, H.G.	1956
Colburn, Burt	1970
Commander, C.C.	1952
Cooper, W.C.	1981
Dickey, R.D.	1968
Edsall, R.S.	1967
Etxeberria, Ed	2016
Everett, Paul H.	1986
Fairchild, David	1922
Fifield, Willard M.	1955
Flagler, H.M.	1903
Floyd, Bayard F.	1944
Floyd, W.L.	1939
Ford, Harry	1985
Forsee, W.T., Jr.	1973
Gaitskill, S.H.	1909
Gardner, Frank E.	1967
Garrett, Charles A.	1951
Goldweber, Seymour	1984
Grierson, William	1979
Guzman, Victor L.	1987
Haden, Mrs. Florence P.	1934
Hall, David J.	2011
Harding, Paul L.	1968
Hart, W.S.	1909
Hastings, H.G.	1939
Hatton, Thurman T.	1987
Hayslip, Norman C.	1981
Henricksen, H.C.	1939
Holland, Frank L.	1962
Holland, Spessard L.	1945
Hoyt, Avery S.	1950
Hoyt, R.D.	1914
Hubbard, E.S.	1922
Hume, H. Harold	1927
Ismail, Mohamed A.	1996
Jackson, Larry K.	2000
Jamison, F.S.	1962
Johnson, Warren O.	1965
Jones, John Paul	1997
Kender, Walter J.	2000
Knight, Robert J., Jr.	2006
Koo, R.C.J.	1978
Krezdorn, A.H.	1979
Krome, William H.	1973
Krome, William J.	1927
Krome, Mrs. Isabelle B.	1960
Lawrence, Fred P.	1973
Lipsey, L.W.	1924
Locascio, Salvadore J.	1996
Logan, J.H.	1965
Lynch, S. John	1975
MacDowell, Louis G.	1968
Magie, Robert O.	1977
Mathias, A.F.	1972
Matthews, Richard F.	1992
Mayo, Nathan	1940
McCornack, A.A.	1986
Menninger, Edwin A.	1964
Miller, Leon W.	1972
Miller, Ralph L.	1972
Montelaro, James	1985
Morton, Julia F.	1989
Mounts, M.U.	1958
Mowry, Harold	1950
Murdock, Del I.	1984
Newell, Wilmon	1940
Norman, Gerald G.	1967
Norris, Robert, E.	1962
O'Byrne, Frank M.	1962
Overman, A.J.	1988
Painter, E.O.	1909
Peterson, J. Hardin	1950
Pratt, J.B.	1980
Redmond, D.	1893
Reed, R.R.	1970
Reitz, Herman J.	1970
Reitz, J. Wayne	1955
Robinson, T. Ralph	1942
Rolfs, P.H.	1921
Rolfs, Mrs. P.H.	1921
Ruehle, George D.	1958
Sargent, Steven A.	2018
Saunders, Fred	1999
Sharpe, Ralph H.	1974
Shaw, Miss Eleanor G.	1927
Sherman, Wayne B.	2003
Showalter, Robert K.	1984
Singleton, Gray	1962
Skinner, L.B.	1931
Sloan, G. Dexter	1964
Smith, Paul F.	1972
Smoot, John J.	1986
Spalding, Donald H.	1987
Spencer, E.L.	1962
Stamps, Robert	2010
Steffani, C.H.	1958
Stephens, James M.	1995
Stevens, H.B.	1934
Swingle, W.T.	1941
Syvertsen, James P	2013
Taber, George L.	1914
Tait, W.L.	1941
Talbott, George M.	1980
Tenny, Lloyd S.	1956
Thompson, Ralph P.	1962
Thompson, W.L.	1962
Thullbery, Howard A.	1962
Todd, Norman	1991
Tucker, David P.	1999
Veldhuis, M.K.	1972
Ward, W.F.	1962
Waters, Will E.	1997
Webber, H.J.	1941
Wedgworth, Ruth S.	1965
Wenzel, F.W.	1973
Wheaton, T. Adair	1994
Wilfret, Gary J.	1998
Wilson, Lorenzo A.	1934
Wiltbank, William J.	1987
Winsberg, Theodore W.	2007
Winston, J.R.	1960
Wolfe, H.S.	1964
Young, T.W.	1978
Yothers, W.W.	1976
Ziegler, L.W.	1976

**Date listed is the year in which the award was received.*

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1993	Michael O. Taylor
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1998	Larry E. Beasley
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2002	William S. Castle
2003	Jonathan H. Crane
2004	Craig Campbell
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2006	Jacqueline K. Burns
2007	Peter McClure
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2010	Jeffrey K. Brecht
2011	Richard Tyson
2012	Juanita Popenoe
2013	Nancy Roe
2014	Ed Etxeberria
2015	Steve Sargent
2016	Chris Oswalt
2017	Mark Ritenour
2018	Eric Simonne
2019	Gene McAvoy
2020	Jeffrey Williamson

Outstanding Growers or Commercial Horticulturists

1997	Norman Todd	Grove Crafters, Labelle, FL
1998	Ted Winsberg	Green Cay Farms, Boynton Beach, FL
1999	Larry K. Jackson	Horticultural Consultant, Auburndale, FL
2000	Gary E. Zill	Zill High Performance Plants, Boynton Beach, FL
2001	Murray J. Corman	Garden of Delights, Davie, FL
2002	Craig Campbell	Valent Biosciences, Orlando, FL
2003	Derek Burch	Masterworks, Plantation, FL
2004	Scott Emerson	Citrus & Vegetable Magazine, Tampa, FL
2005	James "Buster" Pratt	Hanes City, FL
2008	David J. Hall	HDH Agri Products, Tavares, FL
2009	Kenneth Shuler	Stephen's Produce, Jupiter, FL
2010	Peter McClure	Evans Properties, Okeechobee, FL
2011	Michael Edenfield	Bayer CropScience, Windemere, FL
2012	Robert T. McMillan, Jr.	Kerry's Bromeliads Nursery Inc., Homestead, FL
2013	Alvin Cheng	JBT FoodTech, Lakeland, FL
2014	*not awarded*	
2015	*not awarded*	
2016	Steve Rogers	Ecostat Inc., Highland City, FL
2017	Noris Ledsesma	Fairchild Tropical Bodanic Garden, Homestead, FL
2018	Erin Harlow	Dubal County Extension, Jacksonville, FL
2019	*not awarded*	
2020	Noris Ledesma	Miami, FL

FSHS Annual Meeting Host Cities

1888	Ocala
1889	Orlando
1890	De Land
1891	Interlachen
1892	Ormond Beach
1893	Pensacola
1894	Jacksonville
1895	Jacksonville
1896	Jacksonville
1897	Orlando
1898	Orlando
1899	Jacksonville
1900	Jacksonville
1901	St. Augustine
1902	Tampa
1903	Miami
1904	Jacksonville
1905	Jacksonville
1906	Jacksonville
1907	St. Petersburg
1908	Gainesville
1909	Daytona
1910	Orlando
1911	Jacksonville
1912	Miami and Havana, Cuba
1913	DeLand
1914	Palatka
1915	Tampa
1916	Arcadia
1917	Arcadia
1918	Ft. Myers
1919	Orlando
1920	Ocala
1921	Miami
1922	Lakeland
1923	Orlando
1924	Tampa
1925	Eustis
1926	Cocoa Beach
1927	Bradenton
1928	Winter Haven
1929	Clearwater
1930	Sebring
1931	Miami and Homestead (spring meeting) Mariana (fall meeting)
1932	Gainesville
1933	Lake Wales
1924	Orlando
1935	Vero Beach
1936	DeLand
1937	Ocala
1939	Winter Haven
1939	Hollywood
1940	Tampa
1941	Orlando
1942	West Palm Beach
1943	Winter Haven
1944	Winter Haven
1945	Orlando
1946	Miami
1947	St. Petersburg
1948	West Palm Beach
1949	Tampa
1950	Winter Haven
1951	West Palm Beach
1952	St. Petersburg
1953	Daytona Beach
1954	Miami Beach
1955	Clearwater
1956	Orlando
1957	Miami Beach
1958	Clearwater
1959	Miami
1960	Tampa
1961	Miami Beach
1962	Miami Beach
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1966	Miami Beach
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1969	Miami Beach
1970	Miami Beach
1971	Miami Beach
1972	Miami Beach
1973	Miami Beach
1974	Miami Beach
1975	Lake Buena Vista
1976	Miami Beach
1977	Lake Buena Vista
1978	Miami Beach
1979	Lake Buena Vista
1980	Miami Beach
1981	Lake Buena Vista
1982	Miami Beach
1983	Daytona Beach
1984	Miami Beach
1985	Tampa
1986	Miami Beach
1987	Orlando
1988	Miami Beach
1989	Tampa
1990	Lake Buena Vista
1991	Miami Beach
1992	Tampa
1993	Miami Beach
1994	Orlando
1995	Orlando
1996	Orlando
1997	Orlando
1998	St. Petersburg
1999	Stuart
2000	Lake Buena Vista
2001	Stuart
2002	Marco Island
2003	Orlando
2004	Orlando
2005	Tampa
2006	Tampa
2007	Palm Beach Gardens
2008	Ft. Lauderdale
2009	Jacksonville
2010	Crystal River
2011	St. Petersburg
2012	Delray Beach
2013	Sarasota
2014	Clearwater
2015	St. Augustine
2016	Stuart
2017	Tampa
2018	Fort Lauderdale
2019	Maitland
2020	Online via UF TEAMS

2020 Florida State Horticulture Society Keynote Address

Expanding Our Plant Palette: The Role of Native and Non-invasive Cultivars

Sandra B. Wilson*

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Additional index words. invasiveness, ornamentals, propagation, variety trials

Ornamental horticulture has been recognized as the fastest growing segment of U.S. agriculture, as well as the main source of plant invasions worldwide. Florida ranks second among U.S. states in the degree of ecosystem devastation as a result of exotic invasive species. In the past two decades in Florida, tremendous advances have been made to identify and/or select non-invasive improved forms of a number of popular ornamental species such as Chinese privet (*Ligustrum chinensis*), fountain grass (*Pennisetum setaceum*), heavenly bamboo (*Nandina domestica*), lantana (*Lantana camara*), maiden silvergrass (*Miscanthus sinensis*), Mexican petunia (*Ruellia simplex*), porterweed (*Stachytarpheta cayennensis*), and trailing lantana (*Lantana montevidensis*). In addition to promoting superior, research-based, non-native cultivars or selections as viable alternatives to invasive ornamental plants, much progress has been made to identify/propagate and/or select ornamental native species that can also serve as suitable alternatives. Florida boasts abundant richness in native flora with over 3,300 native plant species, yet less than a quarter of these are in cultivation. When used correctly, native plants can naturally offer desired aesthetic attributes such as color and form, while bringing biodiversity and function to sustainable landscapes and gardens. While propagation protocols are lacking for many native species with ornamental value in Florida, significant progress has been made in the propagation, production, and landscape trialing of a number of species such as sandhill milkweed (*Asclepias humistrata*), squareflower (*Paronychia erecta*), coastalplain honeycombhead (*Balduina angustifolia*), wireweeds (*Polygonella* sp.), goldenasters (*Chrysopsis* sp.), wild coffees (*Psychotria* sp.), and sweet acacia (*Vachellia farnesiana*), to name a few. Undeniably, opportunities exist for better consumer awareness, marketing and promotion of environmentally friendly plants that can offer similar form, flowering, fruiting, and growing requirements (sun, soil, moisture) as popular invasive exotics.

Throughout time, plant exploration and introduction have played integral roles in U.S. agriculture. While the majority of introduced ornamental plants do not escape cultivation, some plants spread into natural areas, develop self-sustaining populations, and subsequently disrupt function and form of natural ecosystems (van Kleunen et al., 2018). Through the U.S. Executive Order 13112, an invasive species is defined as an alien species whose introduction does or is likely to cause economic or environmental harm or harm to human health [U.S. Department of Agriculture, National Invasive Species Information Center (USDA, NISIC), 2021)]. This is a global issue with worldwide efforts underway to increase our understanding of invasion biology (Dai et al., 2020), management/control (Strgulc Krajšek et al., 2020), and risk/prevention (Bayón and Vilà, 2019; Conser et al., 2015).

Alien (exotic non-native) species are thought to comprise as much as 80% of the plant inventory held by U.S. nurseries (Hulme et al., 2018). Traits that might be economically beneficial to a nursery professional such as disease/pest resistance, uniform germination and plant growth, and high fertility are traits that could also increase invasive potential (Anderson et al., 2006). The probability of plants becoming naturalized increases significantly with the number of years the plants were marketed and their ornamental value (Pemberton and Liu, 2009). Hence, unintentionally but indisputably, the ornamental horticulture industry has long been the primary source for invasive plants; and this is a targeted issue of many countries (Hulme et al., 2018; Pyšek et al., 2011; van Kleunen et al., 2018).

In the past decade, significant progress has been made by the ornamental industry to minimize the risk of invasive plant introductions. Voluntary codes of conduct have been adopted nationally by botanic gardens and the horticulture trade to help reduce the pathway of invasive plants (Heywood, 2014). Regional results of such efforts are promising; as a recent survey revealed that of the 6885 taxa grown by mid-Atlantic nurseries, only 4% were considered invasive in these respective states (Coombs et al., 2020). Simultaneously, plant breeders have been looking for and developing new cultivars with much reduced or eliminated invasive potential that can replace invasive ones (Li et al., 2004; Vining et al., 2012). These efforts are favorably supported by industry professionals, as a 2015 survey of the southeastern nursery industry reported 74% of participants expressed a positive opinion of sterile cultivar research (Bechtloff et al., 2019) and a willingness to share information about alternatives to invasive species with their customers (Coats et al., 2011). Still, it can be acclaimed that attitude change alone is simply not enough to curtail landscape use of invasive ornamentals, as newly released cultivars are largely not subject to invasive screening or introduction fees (Barbier et al., 2013).

Funding support is greatly acknowledged from the Florida Department of Agriculture and Consumer Services (FDACS) Specialty Crop Block Grant Program, the Florida Fish and Wildlife Conservation Commission, the Center for Applied Nursery Research, the Florida Wildflower Foundation, and the Florida Nursery Growers and Landscape Association Endowment Fund.

This paper was originally presented at the 2020 meeting of the Florida State Horticultural Society

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The Situation in Florida

Florida has the second largest ornamental industry in the country with total annual industry sales estimated at $10.7 billion (Hodges et al., 2016). Significant efforts have been made to accurately assess and predict the invasiveness of some exotic plant species commonly grown in Florida (Fox and Gordon, 2009). The Florida Department of Agriculture and Consumer Services (FDACS) is the only agency with regulatory authority to prevent the sale and distribution of invasive plants in the state (FDACS, Division of Plant Industry, 2021). Yet, it is often too late for effective control once a plant species makes it to a governed noxious weed list. Many of the ornamentals listed as invasive by Florida's Exotic Pest Plant Council (FLEPPC, 2021) or University of Florida/ Institute of Food and Agricultural Sciences' (UF/IFAS') *Status Assessment on Non-native Plants in Florida's Natural Areas* (UF/ IFAS Assessment, 2021) are still in commercial production as cultivated forms that differ from the wild type or resident taxon (Bechtloff et al., 2019). In Florida, if a species is designated as invasive, all cultivars fall under this classification unless proven otherwise through an internally approved UF/IFAS Infraspecific Taxon Protocol (IFTP) Evaluation. This protocol consists of 12 questions to determine 1) if the taxon displays invasive traits that cause greater ecological impact than the wild type or resident species and if it can be readily distinguished; and 2) the fecundity of the taxon and its chances of regression or hybridization to characteristics of the resident/wild type species (Lieurance, 2016). Consequently, all new ornamental cultivars (i.e., those closely related to an invasive resident taxon) proposed for release by UF breeders are subjected to this invasive screening prior to approval.

Identifying exotic non-invasive cultivars as alternatives to invasive species

Over the last two decades, UF researchers have been working to determine the invasive potential of nearly 20 ornamental species and their cultivars, including popular landscape plants such as trailing lantana (*Lantana montevidensis*) (Wilson et al., 2020), porterweed (*Stachytarpheta cayennensis*) (Qian et al., 2021; Wilson et al., 2009), butterfly bush (*Buddleja davidii*) (Wilson et al., 2004), Chinese privet (*Ligustrum sinense*) (Fetouh et al., 2020), fountain grass (*Pennisetum setaceum*) (Wilson and Knox, 2009), maiden silver grass (*Miscanthus sinensis*) (Wilson and Knox, 2006), and heavenly bamboo (*Nandina domestica*) (Wilson et al., 2021). In addition, as part of planned breeding programs, UF breeders have developed genetic and molecular techniques to reduce the fecundity of plants, leading to numerous sterile cultivars of Mexican petunia (*Ruellia simplex*) (Freyre et al., 2016) and lantana (*Lantana camara*) (Deng et al., 2020). As a result of these efforts, the invasive wild type forms are gradually being replaced with newer non-invasive, UF-IFAS IFTP-approved cultivars that are superior in flowering and performance. Even without planned breeding, sterility is not uncommon to find among cultivars of a number of species. To illustrate, Wilson et al. (2021) claimed nearly three-fourths of the 25 heavenly bamboo cultivars evaluated were non-fruiting, meriting consideration for use. In another study, porterweed selections were found to vary considerably in their chromosome number, pollen stainability, and nuclear DNA content (Qian et al., 2021), with half of the selections evaluated having high female sterility (Wilson et al., 2009).

Despite much progress in the identification of safe non-invasive alternatives, disagreement remains about the level of fecundity in cultivars that can be tolerated without posing a risk to the environment. Bufford and Daehler (2014) caution that horticultural selection for sterility (i.e., induced through transgenic techniques, through interspecific hybridization, or through chemically induced polyploidy to create triploid plants) can yield low-risk sterile cultivars of popular ornamentals provided that further hybridization or allopolyploidy does not restore fertility and vegetative spread is limited. Knight et al. (2011) question how much of a reduction in seed production or seed viability is necessary to create a cultivar that will not be invasive in natural areas; and emphasize that reduced seed production may be insufficient to eliminate the invasive potential of a species. Some examples of policy regulating the use of non-invasive cultivars do exist. For instance, the Oregon Department of Agriculture (ODA) approved seedless cultivars of a noxious weed, butterfly bush, for propagation, transportation, and sale provided they produce less than 2% viable seeds (ODA, 2021). Cultivars have also been exempted at the state level. For example, FDACS exempted two cultivars of a noxious weed, Chinese privet: one variegated ('Variegatum') and one chartreuse ('Sunshine') (FDACS, 2021). Yet, concern lingers due to the ability chimeral privet cultivars to revert back to their wild type forms (Wilson et al., 2014). Other concerns have been raised about the vegetative aggression some species exhibit, such as Mexican petunia. While new non-fruiting cultivars such as 'R10-102' (Mayan Purple), 'R10-105-Q54' (Mayan Pink), and 'R10-108' (Mayan White) were approved for use in Florida, cautionary restrictions have been applied due to their risk of vegetative spread (UF IFAS Assessment, 2021). Recently, Datta et al. (2020) reviewed the biological basis of sterility and methods used to generate and confirm sterile cultivars. Noteworthy, are questions they put forward when considering the risk of cultivar invasions; including, what the trait differences are between the proposed safe alternatives and corresponding invasive species, and whether these differences are spatially and temporally stable. Thus, it cannot be overstated that the processes of non-invasive cultivar development that employ forms of genetic mutation and traditional breeding are complex and warrant rigorous scrutiny and screening prior to introduction.

It seems plausible that filling the market with low to no-fruiting cultivars as alternatives to non-regulated (not listed by FDACS) invasive wild type species can help prevent further spread. However, consumer education in distinguishing between recommended and non-recommended cultivars is lacking. Furthermore, while significant strides have been made towards promotion of non-invasive plant use, there is little information available on what economically feasible alternatives are suitable and easy to purchase. Alternative lists for north (Knox et al., 2018a), central (Knox et al., 2018b) and south Florida (Knox et al., 2018c) are available that present native and non-native substitutes for common invasive ornamentals. However, only a limited number of sterile non-invasive cultivars have been released and they are largely unknown on a national scale. Sterile non-invasive cultivars are typically not labeled as such in retail markets, despite research that shows informed labeling may increase a consumer's willingness to buy these plants (Yue et al., 2011).

Confusion among consumers on responsible plant selection and use is echoed by industry interest in making informed decisions on the plant inventory they offer. The voluntary or involuntary regulation of existing invasive nursery inventory is complicated. Drew et al. (2010) point out that the plant availability market is largely driven by consumers who need access to reliable information about the plants they are buying. Dehnen-Schmutz and Conroy (2018) suggest using a citizen's science approach where

gardeners are asked to report ornamental plants that are spreading and difficult to control in their landscapes. Clearly, much can be gained from scientists, consumers, and industry professionals working together to identify solutions that help decrease the spread of invasive species.

Using native plants as alternatives to ornamental invasive species

As the ornamental invasive plant issue continues to escalate, some look to natives as obvious options, to avoid the risk of exotic plant invasion. In fact, the popularity of native landscaping has surged in the last decade as more and more consumers recognize the value in sustainable gardens. Native plants have long been admired for their tolerance of a range of conditions, water-use efficiency (Antunes et al., 2018; Cavaleri and Sack, 2010), and tremendous resource value to diverse pollinators (Campbell et al., 2017; Ricker et al., 2019; Seitz et al., 2020). Studies have shown that customers are willing to pay more money for native plants compared to their non-native counterparts (Yue et al., 2011) and even a 14% price premium for pollinator friendly landscape plants (Khachatryan et al., 2017). Yet it is estimated that three fourths of native flora is not in cultivation, representing only 15.9% of total nursery sales in Florida (Hodges et al., 2016). White et al. (2018) identified a little more than 800 active native vendors selling about 26% of all US native flora. Similarly, in Florida we estimate that less than 25% of our 3,300 native plant species are in cultivation (FANN, 2021; Wunderlin et al., 2021). Thus, great opportunity exists to increase our native plant palette by evaluating whether native plants that are ornamentally attractive in their natural settings can adapt to our modified urban landscapes and gardens.

Major challenges for the promotion of native plants for wider landscape uses typically include: a lack of species-specific propagation protocols; necessary consumer and industry education (Kauth and Perez, 2011); and identification of native plant candidates that are not only aesthetically pleasing and ecologically functional but also economically viable. To these accounts, encouraging progress has been made as reflected by new niche markets for native plants, genotype trialing and selection (Wilde et al., 2015), research-based determination of efficient and reliable propagation by seed, cuttings, and micropropagation (Smith et al., 2014; Trigiano et al., 2018; Campbell et al., 2021), and the establishment of a central, online repository of propagation resources that includes a searchable database (RNGR, 2021).

In summary, a diverse palette of native and nonnative plant options exists as viable alternatives to ornamental invasives worldwide. As our ornamental industry continues to evolve and adapt to emerging issues to stay competitive, horticultural research, teaching and outreach will undoubtedly remain integral to advancing sustainable landscapes and gardens.

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Evaluation of Vigor of Four Mango Cultivars In South Florida

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Mango (*Mangifera indica*) in South Florida is primarily grown in Miami-Dade, Lee and Palm Beach Counties. There is an increasing demand for dwarf and semi-dwarf mango cultivars in South Florida because they are more efficient and less likely to suffer wind damage. Mango tree vigor appears to be controlled by genotypes, although climate and other environmental factors can influence their growth habit. This study evaluated four mango cultivars: 'Angie', 'Mallika', 'Nam Doc Mai', and 'Esmeralda'. The trial was conducted at a private mango farm in the Redland farming district of South Florida. The results are preliminary and include data for 2018 and 2019. The variables measured were height of the tree, stem or trunk diameter, canopy volume, and growth flows. Additional analysis of the xylem and phloem cells of the mango varieties were compared. The results show significant differnces in vigor of the cultivars in the study. 'Angie' seemed to have considerably lower vigor than the other cultivars evaluated.

The mango industry in Florida started more than 100 years ago. Today, mango is grown primarily in south Florida. In 2013, Florida's mango acreage was principally located in Miami-Dade County with 600 acres. The remaining mango acreage is located in Lee, Palm Beach, and other counties with an appropriate climate. (Ledesma, 2015). Farms in Dade County are a mix of urban and rural locations, with sizes ranging from 1/4 of an acre to almost 30 acres. Growers' farming experience varies greatly. Some are very experienced at growing other crops such as avocado, longan, mamey sapote, guava, etc. There are others who have no prior experience with farming. The smallest farms are usually in grower's backyards that have been turned over to their farming efforts. The mango continues to grow in importance in the local market due to interest in the local food movement and the poor quality of imported mangos. These factors have led to an interest in new mango plantings in Miami-Dade and other Florida counties.

For small-scale specialty mango production in south Florida, growers must be actively seeking alternative markets to increase their profitability. There are increasing opportunities for mango production using innovative production systems and new cultivars that can readily be distinguished from commercially available ones and will allow for better prices and profit (Ledesma and Campbell, 2017).

Three major aspects of tree vigor control in mango are genetic selection, rootstock/interstock combinations, and chemical growth regulators. Publications from other regions discuss these (Rashedy et al., 2014). Additional factors contributing to mango tree vigor are grafting height, method of propagation, type of rootstock, planting density, training and pruning, regularity of bearing, intercroping, climate and other environmental factors such as soil, water, diseases and pests, etc. This paper examens how these factors affect the 'Angie', 'Mallika', 'Nam Doc Mai', and 'Esmeralda' mango cultivars.

Methodology

This study evaluates four mango cultivars: 'Angie', 'Mallika', 'Nam Doc Mai', and 'Esmeralda'. The trial used10 mature trees per cultivar with an average age of 5 years. The evaluation was conducted from March through September of 2018 and 2019.

Location. The study was conducted at a private mango farm in the Redland farming district of Miami-Dade County in south Florida. The orchard consists of 1.5 acres with a spacing of 4 m within rows and 6 m between rows. The experimental site consisted of a crushed oolitic limestone substrate that had been rock-plowed for several decades, but left fallow for the past 20 years. The trees are grafted on 'Turpentine' rootstock, and have been hand pruned every year after harvest, except for the years of evaluation, 2018 and 2019. Fertilization and disease control were minimal, using a low input of chemicals and a reliance on hand labor.

Variables. Vigor is defined as a visual estimate of the synthetic growth summary. The variables measured to estimate vigor consider: the height of the tree, stem diameter, canopy volume (Vázquez-Valdivia et al., 2005), and growth flows (El-Khawaga and Maklad, 2013). Tree measurements were recorded, and the trees separated into different parts.

Stem diameter. Using a graduated tape and measuring the circumference of the stem.

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Canopy volume. Estimated assuming that it is equivalent to the area of the circumference defined by the average canopy diameter.

V= canopy volume
D= diameter of canopy base (m)
H= canopy height (m)

Vegetative growth flow. In mango, vegetative growth does not occur continuously, but occurs in the form of growth flows that end when the new leaves have fully expanded (Davenport, 2007). In Florida, a vegetative flow is usually followed by a rest period which is relatively short in young trees, but can last more than eight months in adult trees (Davenport, 2007). The number and frequency of vegetative flows per year depends on the cultivar, availability of soil moisture and volume of the previous crop, although in each outbreak there can be three to four growth flows per year (Davenport, 2007). For 'Angie' only the measurement of the first flow was performed, for the varieties 'Nam Doc Mai' and 'Mallika' the measurement of two vegetative growth flows was performed, and for a control, four vegetative growth flows were measured.

Analysis of the xylem and phloem cells. *S*amples of the flows of the different studied varieties were collected, fixed in formaldehyde, alcohol, acetic acid. Sample dehydration samples was done using increasing concentrations of ethyl and butyl alcohols. Then the samples were infiltrated with paraffin wax. The samples were sectioned crosswise with a rotating microtome and stained with safranin (red staining of lignified cell walls) and light green (blue-green cellulose wall staining) and examined with an optical microscope.

Results and Discussion

The results are preliminary and include data for 2018 and 2019. The evaluation began in March 2018. The vigor parameters were calculated for each cultivar. Average vegetative growth flow for 'Esmeralda' was measured 4 times per year, 'Nam Doc Mai' and 'Mallika' twice, and 'Angie' once (Fig. 1). Growth (length) varied from 3.8 to 9.2 inches, with 'Angie' being the shortest with an average of 3.9 inches. 'Mallika' and 'Nam Doc Mai' had similar average lengths of 6.9 inches while 'Esmeralda' had an average 8.1 inches. The analysis of the variance showed that vegetative growth flow was highly significant among the studied cultivars ($P < 0.001$).

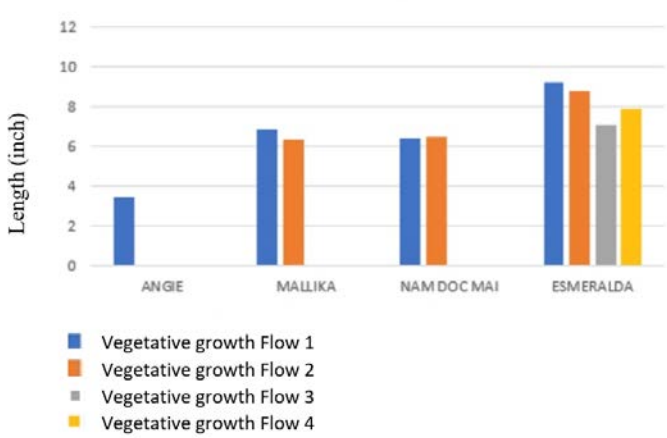

Fig.1 Vegetative growth flow for four cultivars.

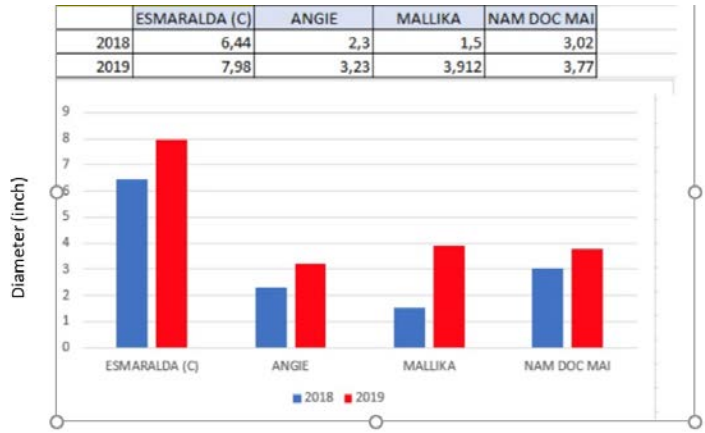

Fig. 2. Stem diameter of four cultivars.

Stem diameter also varies among cultivars. 'Esmeralda' had the largest trunk diameter at 6.44 inches for first year and 7.98 inches for the second. 'Nam Doc Mai' had trunk diameters of 3.02 inches for 2018 and 3.77 inches for 2019. 'Mallika' showed with rapid growth with the trunk diameter increasing from 1.5 inches in 2018 to 3.91 inches in 2019. 'Angie' had trunk diameters of 2.3 inches in 2018 and 3.23 inches in 2019 (Fig. 2).

The canopy volume also varies between cultivars. 'Esmeralda' had the largest canopy with 1843 ft^3 for 2018 and 3959 ft^3 in 2019. 'Mallika' had 225 ft^3 in 2018 and 445 ft^3 in 2019; 'Nam Doc Mai' had 204 ft^3 in 2018 and 376 ft^3 in 2019; and 'Angie' had the smallest canopy at 81 ft^3 in 2018 and 232 ft^3 in 2019 (Fig. 3). The analysis of the xylem and phloem cells samples of the flows of the different varieties studied had not enough data for a conclusion.

From this study, we found that 'Angie' had the least vigorous growth, followed by 'Nam Doc Mai', then 'Mallika', with 'Esmeralda' being the most vigorous. 'Angie' had significantly less vigorous growth when compared with the other cultivars.

If one makes the assumption that cultivars with low to moderate vigor are more easily managed to keep them small while maintaining good fruit production, 'Angie' would be considered a desirable fruit tree for south Florida.

Future evaluations of fruit set are recommended to establish yield versus vigor using the mango cultivars from this study

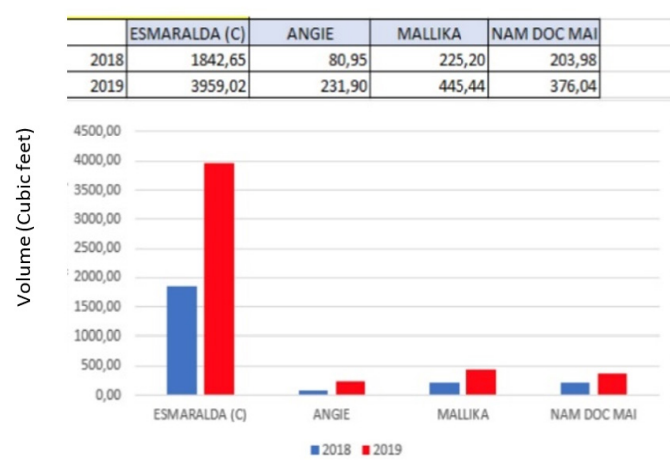

Fig. 3. Canopy volume.

under south Florida conditions. In the present study, trees were tipped one year before the study. We suggest that the trees be left unpruned for future experiments on vigor.

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Current Recommendations for Control of Laurel Wilt and Its Ambrosia Beetle Vectors In Florida's Commercial Avocado Groves

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Laurel wilt (LW) is a lethal vascular wilt disease of woody plants in the Lauraceae family, caused by the fungus *Raffaelea lauricola* (*Rl*). Introduced into the U.S. in 2002 and detected in Florida's avocado production area in 2012, LW has caused the death of more than half a billion redbay trees and 140,000 avocado trees worth an estimated $46.2 million. *Raffaelea lauricola* is dependent on ambrosia beetles (AB) for dispersal. In avocado groves *Rl* is associated with several native and exotic ambrosia beetles. These beetles occur in large numbers and usually colonize trees that are physiologically stressed. After trees are inoculated by AB, *Rl* can spread through root grafts. Long-distance spread of the disease is by movement of beetle infested wood products. To date, no avocado cultivars (scions or rootstocks) have shown tolerance to *Rl*. Symptoms of LW begin as green leaf wilting in one or more sections of the canopy, followed by leaf desiccation, stem and limb dieback, and ultimately tree death; frass tubes are a sign that AB have bored into the tree. The pathogen moves rapidly through the tree resulting, in obstruction of the water conducting tissue (xylem) caused by the host tree's attempts to contain the spread of the fungus by forming barriers/eliciting the formation of tyloses. To date there is no cure for the disease. Current recommendations to contain the disease include early detection, complete tree removal and destruction (chipping) of infected trees and then applying insecticides to the trunk of trees within one acre of the removed trees to reduce AB populations. Prophylactic fungicide injections, applying formulations of the biological mycoinsecticide *Beauveria bassiana* (entomopathogen) to the entire orchard in late winter/early spring, and pruning to improve light levels within the tree canopies, are also recommended. In the absence of cost-effective control measures for LW, the current strategy to maintain avocado production in South Florida is to replant trees that are lost to the disease and to keep investing in research to find a long-term and sustainable solutions.

The laurel wilt-ambrosia beetle complex continues to threaten Florida's avocado industry despite 14 years of research to find an entirely effective control program. To date the loss of over 140,000 commercial avocado trees can be attributed to LW worth an estimated $46.2 million (Evans and Crane, 2019). This is because little to nothing was known about the fungal phytopathogenic symbiont, *Raffaelea lauricola* (*Rl*; causal agent of the disease) when it was introduced into the United States in 2002 and the unexpected lateral transfer of *Rl* from the initial ambrosia beetle vector, *Xyleborus glabratus,* to at least nine other ambrosia beetle species that are the primary carriers of the pathogen in avocado systems (Carrillo et al., 2014; Ploetz et al., 2017). Large populations of these beetles commonly inhabit avocado groves and three species, *X. bispinatus*, *X. volvulus* and *Xyleborinus saxeseni* have been implicated in transmission of the pathogen to avocado trees (Carrillo et al., 2014). These beetles have a short flight period of ~1 h before sunset and usually colonize trees that are physiologically stressed (Menocal et al., 2018). The rapid movement of the pathogen within and among adjacent root-grafted avocado trees, the rapid rate of decline (4–8 weeks after symptoms onset) of avocado trees in response to the pathogen, and the ability of ABs to reproduce rapidly well protected inside the trees, have made development of economically feasible control tactics difficult (Inch et al., 2012; Inch and Ploetz et al., 2011a; Ploetz et al., 2011b; Ploetz et al., 2015). Additional challenges include the proximity of natural areas harboring LW susceptible hosts and back yard avocado trees serving as reservoirs for the pathogen and routes of dispersal among avocado groves.

This document updates previous recommendations for control strategies and mitigation of LW (Crane et al., 2008; Mayfield et al., 2008; Wasielewski et al., 2014; Crane et al., 2016; Wasielewski et al., 2016; Wasielewski and Crane, 2017; Ploetz et al., 2017) and expands on current recommendations (Crane et al., 2020a and Crane et al., 2020b).

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Current research findings related to control strategies

The LW pathogen is highly virulent and moves through the xylem rapidly within avocado trees and among mature root-grafted avocado trees. As few as 39 CFU (colony forming units) can induce symptoms of LW and the eventual death of mature avocado trees.

Prophylactic injection of propiconazole (Tilt®) every 12 to 24 months appears to be effective in protecting avocado trees from developing symptoms of LW. However, the time from injection to systemic coverage within a tree takes seven to eight months.

Ambrosia beetles implicated in *Rl* transmission are present year-round and their flight activity increases during late winter–early spring.

Groves with higher light levels have significantly reduced AB abundance compared to groves allowed to form dense canopy resulting in shaded conditions. Pruning (hedging, topping or selective pruning) to maintain higher canopy and grove light levels, re-establishing tree canopies (rejuvenating old non-productive trees) with severe pruning (e.g., hat-racking or stumping trees to four ft) and top-working large trees increase grove light levels and suppress AB activity.

Current control recommendations

Promote and maintain healthy trees through proper fertilizer, irrigation, and pest management. Poorly maintained and environmentally stressed trees (e.g., affected by drought, flood, drought, other diseases, wind/storm damage and/or freeze exposure) are attractive to AB and increase their chances of being colonized by AB carrying *Rl*.

Scout groves to detect trees with early LW symptoms such as green-leaf wilting. The earlier infected trees are removed and destroyed, the more likely the disease outbreak may be contained. When trees are showing symptoms associated to later stages of disease progress (i.e., large segments of the canopy with desiccated foliage), the LW pathogen has probably already moved through root grafts to adjacent health trees.

Rogue (uproot and chip/shred) LW-affected trees immediately upon detection. Severing the root system of adjacent trees is the most important step to limiting the spread of the pathogen to adjacent root grafted trees. In addition, rogueing helps to reduce AB populations by destroying the galleries where they reproduce. Spray the chipped or shredded wood twice with a contact insecticide. Insecticides registered for use on avocado include Malathion, Danitol®, Agri-Mek®SC, Talstar®S, Hero® (nonbearing trees only), Botanigard®, and Mycotrol® (*Beauveria bassiana*).

Two trunk and lower limb directed contact insecticide applications (fifteen days apart) should be made to protect healthy trees within a one-acre area of rogued LW-affected tree(s).

During late winter–early spring, when AB populations and activity increase, apply two grove-wide applications of the mycoinsecticides BotaniGard®ES or Mycotrol® (*Beauveria bassiana*) to suppress AB populations.

Productive groves with the lower canopy intact should be pruned annually to maintain canopy light levels and production. Groves with overgrown trees that have lost their lower canopy should be rejuvenated, i.e., cut back to four to eight feet to re-establish the lower canopy and then maintained at a height no higher than two-thirds the distance between rows. The re-established productive canopy of top-worked trees grafted to alternative cultivars should also be pruned to maintain the new productive lower canopy through regular pruning.

Consider, injecting non-LW affected groves prophylactically with propiconazole (Tilt®). If this is not possible, rogue LW-symptomatic trees and inject all remaining healthy appearing trees. Some loss of these trees may be expected due to the spread of the pathogen prior to injection. When additional avocado trees show LW symptoms, remove them as soon as possible. Injected trees will need to be reinjected on at 12 to 24-month interval depending upon pest pressure.

Current mitigation recommendations

Replant avocado trees lost to LW to establish future fruit production and maintain economic viability (Evans et al., 2010; Mosquera et al., 2015). Movement of the LW pathogen by tree-to-tree root grafting is not an issue in young trees because: it takes many years for root grafts to form; young trees are not a preferred host of AB; and the high light exposure of the area around these trees suppresses AB activity.

On-going and future research

The research into the biology and strategies to control and mitigate the LW epidemic continue. Control tactics that show promise include:

- Underlying biotic and abiotic factors that cause physiological stress on avocado trees making them attractive to ambrosia beetles,
- Push-pull (trap-kill) systems to repel, attract and kill AB,
- Understanding the microbiome of AB and how they may be manipulated to suppress AB populations,
- Cross protection of avocado trees with nonpathogenic species of *Rl* or other less virulent vascular fungi or non-viable *Rl*,
- Search for, document, and monitor healthy productive avocado trees that are positive to *Rl* to better understand how they are surviving the pathogen infection,
- Understanding the physiology scion-rootstock relationship and responses to *Rl*, and
- Understanding the molecular basis for disease development and how it may be manipulated to provide avocado trees tolerance to the pathogen.

Conclusion

The laurel wilt disease complex has posed the most significant challenge to avocado production in the western hemisphere. Understanding the biology of the pathogen and its vectors is in progress with the aim of providing new strategies for avocado producers to control and/or mitigate the effects of this devastating epidemic.

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The Introduction and Utilization of Kasturi to South Florida (*Mangifera casturi*)

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The mango, *Mangifera indica* L. (Anacardiaceae), is the best known and most widely cultivated species in the genus *Mangifera*. There are over 69 *Mangifera* species currently recognized in Southeast Asia, with many species locally rare and/or included on the IUCN Red List of Threatened Species. *Mangifera casturi* (Kasturi) is reported as extinct in the wild, although fruit of *M. casturi* are sold at fruit markets in the south of Kalimatan (part of Indonesia and the southern portion of Borneo). There are three reported introductions of Kasturi in south Florida, including deep purple, green, red, and yellow fruit; two of them are included at the USDA plant introduction state at Chapman Field in Miami, FL. Interviews with the pioneers who introduced *M. casturi* to the United States were conducted to clarify the importance of the accessions. *M. casturi* is a vigorous tree that forms a tight, upright canopy with shiny, dark green leaves which contrast with the bright red new growth. It might be interesting to grow Kasturi in South Florida as a boutique market fruit, due to its adaptability to the local climate and the fact that both blooms and fruit are tolerant of anthracnose. The fruit usually weigh 40 to 50 g. They are fibrous, with a juicy sweet flavor resembling passion fruit and lychee.

The mango continues to grow in importance in local markets due to local food movements and the poor quality of imported mangos. New mango plantings are increasing in Miami-Dade County, FL. In the past 5 years, small-scale specialty mango production in south Florida has been increasing in popularity as growers become more creative, experimenting with new products and exploring new markets. Many new cultivars are now available in south Florida, including early and late season varieties to cover a season from May to September (Ledesma, 2015).

A new group of mango aficionados are investing in this diversity of mangos. Tropical fruit clubs are increasing as the south Florida green market contributes to the breadth of the local food movement. Mango flavor, and seasonality have been some of the biggest issues with currently available mangos. There are several *Mangifera* species reported in south Florida including *M. odorata, M. lalijiwa,* and *M. casturi* (Ledesma, et al., 2015).

In recent years non-*M. indica* species have attracted interest from fruit aficionados. The three species *M. odorata, M. lalijiwa*, and *M. casturi* have been propagated and distributed at the International Mango Festival at Fairchild Tropical Botanic Garden for several years. Today they are growing in backyards and private collections in south Florida. There are two introductions of Kasturi reported in South Florida. This paper reviews the origin of each introduction and *M. casturi* itself.

Origin

Kasturi (*M. casturi*) (Fig. 1) may have originated in a very small area around Banjarmasin in South Kalimantan, Indonesia (southern Borneo) (Campbell and Ledesma, 2007). *M. casturi* has

Fig 1. Senior author with a *Mangifera casturi* tree and fruit.

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been reported as extinct in the wild, although fruit of *M. casturi* are grown and sold at fruit markets in South Kalimatan. In the Americas, *M. casturi* has been reported in private collections and Botanical gardens in Guatemala, Peru, Brazil, Hawaii, Puerto Rico, and Florida. Three introductions of Kasturi have been reported in south Florida. Interviews with the pioneers than introduced *M. casturi* to the United States were conducted to clarify the timing of the accessions.

One Florida introduction was from Hawaii by Frankie's Nursery located in Waimanalo, HI. The tree was collected from Banjarmasin, Borneo, southern Kalimantan, Indonesia (Frankie Sekiya, personal communication, 2020). This tree was propagated by Zill's Nursery in Boynton Beach, FL. and donated to the Fairchild Tropical Botanic Garden (FTBG) Fruit Collection. FTNG made two trees from their original one. They were donated to the USDA collection at Chapman Field in Mar. 2001 as Accession number MIA 36626 (Purple) and in 2003 with accession number MIA 36625 98-2186. Both accessions are listed in the Genetic Resources Information Network (GRIN) database system. The two trees were clonally propagated from same original accession but show differences in productivity and size, perhaps due to being grafted onto different rootstocks. MIA 36626 (Purple), is reported as being a more productive tree than MIA 36625 98-2186, the Green with inferior in quality. The fruit weigh an average of 105 g have a dark green non-waxy skin when immature which turns purple-black with yellowish lenticels at maturity (Ayala et al., 2013).

A second introduction was made by Vivian Murray of Treehouse Nursery formerly in Bokeelia, FL. She brought in budwood of *Mangifera casturi* from the Botanical Ark in Australia (Stephen Brady, personal communication, 2020). The tree was propagated by Stephen Brady and donated to the Naples Botanical Garden and Fairchild Tropical Botanic Garden. Both gardens still have this accession in their collection. It is a vigorous tree that forms a tight, upright canopy with shiny, dark green leaves which contrast with the bright red new growth. The fruit are usually 40–50 g. They are fibrous, with a juicy sweet flavor resembling passion fruit and lychee. The skin is thick and deep purple with few yellowish lenticels. DNA evaluations are underway to determine the differences between the accessions.

Horticultural Remarks

Propagation. *M. casturi* has been of use for propagation as an interstock to graft other species of *Mangifera*, including *M. griffithii, M. lalajiwa,* and *M. merillii* (Campbell and Ledesma, 2007) on *M. indica*. *M. casturi*, has been grafted successfully on 'Turpentine' rootstock and trees have been grown in the field for 14 years and have been shown to be a good rootstock/interstock combination for other species (Campbell and Ledesma, 2013). *Mangifera casturi* is compatible with *M. indica* 'Turpentine, and *Mangifera rubrapetala* rootstocks and vice versa. Preliminary observations show that Kasturi rootstocks under *Mangifera rubrapetala* generate dwarf trees, which are more precocious than trees propagated using *M. indica* 'Turpentine' rootstock. Kasturi has been growing in Guatemala for over 15 years from seeds. They start to bear fruit in four or five years. They are consistently productive every year. Kasturi has polyembryonic seeds. For better germination it is that the husk-like seed coat be removed. Then the bare seeds should be soaked at a temperature of around 30–35 °C for about 6 hours. After soaking, the seeds should be sown in soil or a potting mix (light, sandy soil). Seeds sprout within 1–3 weeks. Young seedlings should be kept in a moderately sunny situation with around 60% of full sunlight.

Breeding. Kasturi has been used in breeding to improve mangos and produce new hybrids with reduced susceptibility to disease which will naturally flower in the tropics with no induction and produce good quality marketable fruit (Ledesma, et al., 2015).

Tree size and fruit production. An *M. casturi* tree can be a large tree reaching up to 20 m high, with a trunk diameter of 100 cm and a dense and spreading canopy (Campbell, 2016; Ledesma and Campbell, 2014). Compared to *M. indica*, Kasturi is adapted to high humidity and wet soils. For planting, soils should be made as fertile as possible. Young trees benefit from mulching. Such amendments improve water-holding capacity, nutrient retention and availability, and soil structure (Fig. 1).

Low humidity is detrimental to the health of young trees. Fertilization is best done as three applications per year of an 8–3–9 or other fruit tree formulation in March, July, and September.

Kasturi trees will form a stately, dense and rounded canopy without proper pruning. Horticultural management is necessary to maintain small, healthy and productive trees. With annual pruning the tree is easily maintained at a height and spread of 6.5 to 2–3 ft, respectively. Pruning should be done once per year following harvest of the main crop, or towards the end of the growing season.

Trees grown in Florida have been reported not to flower consistently (Ayala et al., 2013). However, Kasturi trees grafted on *M. rubrapetala* have been observed consistently producing fruit for the past five years. These trees are considerably smaller and easer to control.

Kasturi is early season and have a fruiting duration of about 30–45 d with maturation dates ranging from mid-May to June(Ayala et al., 2013).

Disease resistance. Kasturi has been reported as resistant to the attack of the oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae) (McQuate et al., 2017). *M. casturi* blooms and fruit are tolerant of anthracnose but can be susceptible to powdery mildew, a problem commonly found in many *M. indica* cultivars (Ledesma et al., 2018).

Utilization

Kasturi has been popular and held in high regard across Indonesia, Myanmar, and Thailand. Traditionally the fruits are grown from seeds and consumed locally (Campbell and Ledesma, 2007). More recently, trees have been cultivated by locals in their backyards or on small farms in south Florida as a curiosity. Fruit is occasionally offered by local vendors and has been gaining popularity over the past few years.

The tree is handsome, with a charming dense canopy with deep green shiny leaves and can be used as a ornamental. The inflorescensce are up to 30 cm long, multiflowered and smell like jasmine and are visited by honeybees (Ledesma, et al., 2018). Of course, one of the best features of this beautiful tree is the fruit. The fruit is a beautiful and unique with a deep purple skin coated with heavy wax that looks blue after harvest. The fruits can be picked up off the ground and allowed to finish ripening indoors. The flesh is sweet with a perfect balance of acidity and sweetness. Kasturi has overtones of passion fruit and lychee. It has some fiber and the fruit are small, nevertheless it is a novelty as a mango which is resistant to anthracnose allowing it to be grown without pesticides.

There are reports that the methanolic extract of *Mangifera casturi* fruit have high antioxidant activity with terpenoid and phenolic compounds, with possible applications as anti-inflammatory supplements (Fakhrudin et al., 2013). Studies also report than the pulp of *M. casturi* has a higher nutritional quality in terms of vitamins and minerals than *Mangifera indica* (Barbosa et al.,2017).

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'Jumbo Kesar' Mango for South Florida

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The air-shipment of mangos from India to the United States during the last 3 seasons has increased interest in the production of Indian mango cultivars in south Florida. 'Jumbo Kesar' is originally from India. The original tree was a descendant of the 'Kesar' mango grown at Dedakyali, a village near the town of Gir, Gujarat state. It was introduced in south Florida in 2008. Since that time, trees and fruit have been evaluated. The tree seems to be adapted to home garden and estate agriculture in south Florida. It has better productivity than other Indian cultivars when grown in south Florida as well as better disease resistance and overall fruit quality. The average fruit weighs 550 g and is greenish yellow with a red blush (when exposed to the sun). The flesh hass a deep yellow almost saffron color, is firm and juicy with a complex coconut flavor. The tree is medium sized and manageable with annual pruning. Size can be maintained at or below six feet while maintaining consistent production. The tree and fruit are somewhat tolerant to wet conditions, although fruit quality suffers when the monsoon comes early. The author recommends making larger test plantings of 'Jumbo Kesar' to further evaluate its suitability in south Florida.

Mangos have been cultivated in India since ancient times and are an important part of the Indian culture and economy. India exports nearly 30 varieties of mangos. Important export cultivars include 'Totapuri', 'Alphonso', 'Dashehri', and 'Kesar'. Mangos have been exported from India to the United States since an irradiation treatment was approved in 2002. Since then, air-shipments of mangos from India to the United States have led to an increased interest in the production of Indian mango cultivars in south Florida. Attractive per-box prices and quality issues associated with the long-distance transport and the necessary postharvest treatment of the imported product have made the production of these cultivars in the Western Hemisphere an opportunity for growers (Campbell and Ledesma, 2013).

There is little reliable information regarding the most suitable locations for the commercial production of Indian mangos in south Florida as they are notorious for their horticultural challenges here. Indian cultivars have been introduced to south Florida over the decades with little success. There is some experience with 'Kesar' mango grown by local farmers in south Florida, but low productivity and large tree size have caused problems. The objective of this paper is to discuss 'Jumbo Kesar' as a candidate cultivar for south Florida, based on observations of 'Jumbo Kesar' trees grown in a local commercial orchard. The trees have been under evaluation for fruit and flower characteristics since they were planted in 2008. The authors recommend further evaluations with more details for growth management and productivity of 'Jumbo Kesar' in south Florida.

'Jumbo Kesar' in south Florida. The observations were recorded for 5 years in a commercial orchard in south Florida, using 20 trees grafted on 'Turpentine' rootstock. The trees are now 6 years old. Additional evaluations of fruit and flower morphology were conducted at the Fairchild Farm mango collection in Homestead, FL.

Origin

'Jumbo Kesar' is a seedling of 'Kesar' mango originally grown in Dedakyali village, Gujarat State. Madhavray Savani, a mango farmer from Mota Bhamodra village, started propagating the tree inspired by the improved size of the fruit. The 'Jumbo Kesar' started gaining popularity in the region, and many farmers started growing it. Today the Savani's family has established the Gir Nature Trust, promoting sustainable farming and helping local farmers create a mango corridor using mangos including 'Jumbo Kesar' for wildlife migration. The tree was cloned and established at the living collection of Reliance Industries located at Jamnagar, Gujarat, and from there it was introduced to south Florida in 2008.

Tree. The tree is medium sized and can be kept at or below 6 ft. without interfering with fruiting with annual pruning. Grafted trees have a spreading growth habit and dense foliage. Mature leaves are dark green while the new growth has a reddish-brown color. The tree and fruit have some tolerance to wet conditions, although fruit quality suffers when rainfall starts early. Pruning must occur after harvesting every year to limit tree size. It is advisable to avoid nitrogenous fertilizers and supplemental watering until flowering has occurred. The tree flushes twice or three times a year, with 13-inch internodes. Trees start bearing fruit at the age of 3–5 years and are productive compared with other Indian mangos.

Flowers. 'Jumbo Kesar' inflorescence (Fig 1.) is an average of 24–26 cm long and has an average of 50% to 70% hermaphroditic flowers. Flowers are produced on terminal inflorescences with

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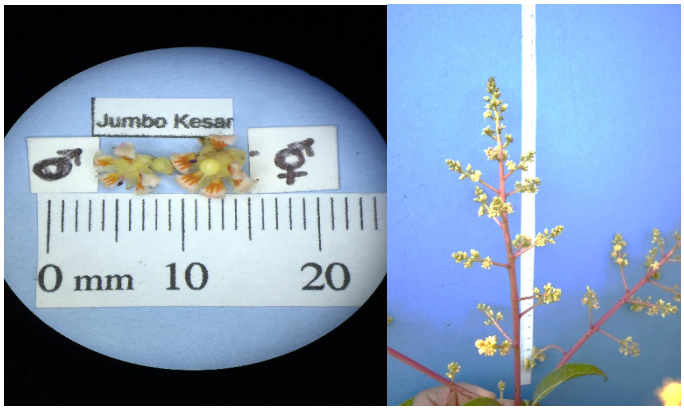

Fig 1. 'Jumbo Kesar' Flowers (left) and inflorescence (right).

thousands of individuals both male and hermaphroditic, which have 5 petals and sepals with a cream color with light violet tones (Ledesma, et al., 2018). Blooming is consistent and generally occurs from December to January depending on temperature. It is better when winters are cool and dry. The tree has a low susceptibility to anthracnose, though powdery mildew can be a problem when relatively high humidity occurs during flowering. 'Jumbo Kesar' requires a spray program for commercial production.

Fruit. 'Jumbo Kesar' has a subtle, floral aroma with components of coconut, honeysuckle, vanilla, and banana. The flavor is excellent and rich with strong notes of coconut, lemon peel, mandarin, lychee, honey, and milk as well as clove and cinnamon. It has a creamy, smooth texture, a Brix of 25%, and a long flavor life in storage. It is a fruit with high overall quality, and is a good alternative for local mango growers and consumers interested in good flavor. Consumer acceptance and eating quality is excellent. The fruit can be marketed to both ethnic and mainstream customers at attractive prices.

The fruit has an average weight of 550 g and is greenish yellow with a red blush when exposed to the sun. The flesh has a deep yellow almost saffron color, and is firm and juicy with small amount of fiber (Fig. 2). Skin is smooth with small, sparse pale-yellow lenticels. It is a monoembryonic cultivar.

Fig 2. 'Jumbo Kesar' fruit.

Maturity and harvesting. The fruit are ready for harvest from June to July in south Florida. Harvesting should be done when the fruit are mature but not ripe. The best flavor is obtained when the fruit is harvested at 25% maturity and stored at room temperature. Postharvest life is short at ambient temperatures, but low temperatures can ruin the flavor.

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Sustainable Strategies to Combat the Papaya Ringspot Virus

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Additional index words. aphid, vector, trap crop, potyvirus, 'Red Lady'

The papaya ringspot virus (PRSV) is a limiting factor for papaya production. All papayas are susceptible to PRSV, except where resistance has been genetically engineered. Studies conducted in Hawai'i suggest that the use of transgenic PRSV-resistant accessions as border rows may prevent aphid-mediated transmission of the virus to susceptible interior plants, potentially expanding the range of varieties that can be cultivated profitably. A field trial to evaluate this technique was carried out at the Tropical Research and Education Center (TREC) in Homestead, Florida. In this experiment, we found that the use of virus-resistant border rows did not meaningfully impede transmission of PRSV to the susceptible cultivar 'Red Lady'.

Viral diseases are a significant impediment to papaya production worldwide, including domestic growing regions of southern Florida, Puerto Rico and Hawai'i. The most significant of these is papaya ringspot virus (PRSV), which dramatically reduces yield and can lead to plant stunting or death. Current strategies to combat PRSV in papaya are limited and consist primarily of copious insecticide applications to control the aphid vector, rogueing and replanting of infected fields, or cultivation of transgenic, PRSV-resistant varieties. Engineered PRSV resistance has been the most effective control strategy, but it has limitations. Fruits of genetically engineered crops can be difficult to market due to the stigma surrounding genetically modified organisms (GMOs). Furthermore, there are few PRSV-resistant varieties available, which limits growers' choices. We therefore sought to expand the range of options available to local papaya growers by evaluating the use of PRSV-resistant border plantings to protect susceptible papaya varieties, an approach recently pioneered by researchers at the USDA-ARS in Hawai'i with promising preliminary results (Matsumoto et al., 2014). The strategy relies on the non-persistent manner in which aphids transmit PRSV. The rationale is that infectious vectors are likely to feed first on resistant plants at the perimeter of the field. In the process, the proboscis and stylus are cleansed of the virus, preventing subsequent transmission. We have evaluated the efficacy of this new PRSV-management technique under southern Florida's distinct growing conditions using regionally relevant papaya accessions.

Materials and Methods

The field trial was established in Spring 2020 at University of Florida's Tropical Research and Education Center (TREC) in Homestead, FL. On 21 Jan. 2020, seeds were sown into trays filled with 1 part Pro-Mix BX Mycorrhizae to 1 part perlite, top-dressed with 14–14–14 slow release fertilizer, and kept in the greenhouse for 9 weeks followed by a one-week acclimation period. Seedlings were transplanted outdoors on 30 Mar. 2020. PRSV-resistant border plants were derived from open-pollinated (OP) seeds of the University of Florida breeding line S40-2228 (Davis and Ying, 2004). 'Red Lady 786 F1 Hybrid' from Known-You Seeds was used as the susceptible cultivar. The field plot consisted of 21 rows of raised beds with 37 plants in each row. Spacing was 5 ft within-row and 12 ft between rows. Liquid 3–0–10 (N–P–K) fertilizer was supplied daily with irrigation at a rate of 0.25 lb N per acre per day for the first 8 weeks, 0.5 lb N per acre per day for the next 4 weeks, and 1 lb N per acre per day subsequently. Glyphosate was applied as-needed to control weeds.

The experiment included two treatments with four replicates each in a randomized row-column design (see Fig. 1 for field diagram). Treatment A consisted of 6 susceptible 'Red Lady' plants surrounded by 3 additional rows of 'Red Lady' seedlings on all sides. Treatment B consisted of 6 susceptible 'Red Lady' plants surrounded by 3 rows of PRSV-resistant OP S40-2228 seedlings. Each replicate plot was surrounded by one additional row of 'Red Lady' plants on all sides. These plants were artificially inoculated with PRSV on 20 July and again on 3 Aug. 2020 to ensure disease pressure throughout the field.

Results

Six months after trial establishment, plants were 5–6 ft tall and bore mature green fruits, but none had yet begun to ripen. Classic PRSV symptoms were evident throughout the field including mosaic, chlorosis, and distorted leaves, as well as ring spots on the surface of green fruits (Fig. 2). The 6 interior plants within each replicate plot were visibly scored for presence or absence of PRSV symptoms on 27 Sept. 2020 (Table 1). PRSV symptoms were apparent on at least 1 of the 6 plants in every replicate, regardless of border row treatment. Overall, more than 40% of monitored plants were visibly infected across both treatments.

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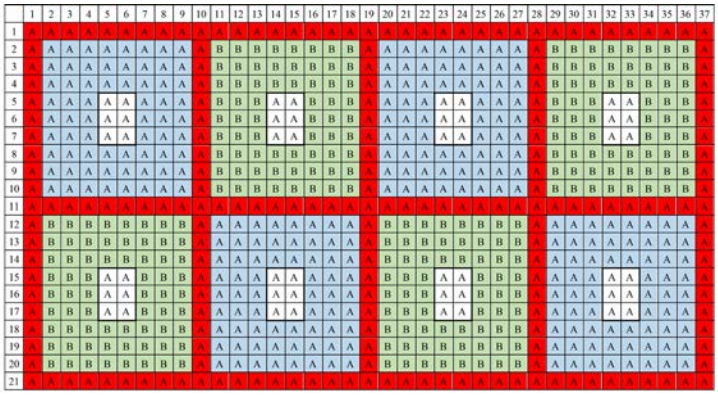

Fig. 1. Field diagram. Each cell represents one papaya plant. 'A' denotes PRSV-susceptible 'Red Lady'. 'B' denotes a PRSV-resistant plant of line OP S40-2228. Blue shading represents Treatment A borders ('Red Lady'). Green shading represents Treatment B borders (transgenic line OP S40-2228). White shading represents the six interior 'Red Lady' plants monitored for PRSV within each plot. Red shading represents artificially inoculated 'Red Lady' papayas.

Fig. 2. PRSV symptoms on leaves (left) and fruit (right) of 'Red Lady' papaya plants.

Table 1. Number of plants with PRSV symptoms after six months. The 6 'Red Lady' plants in the center of each plot were visually scored (presence /absence) for PRSV symptoms. The number of symptomatic plants as of 27 Sept. 2020 is recorded below.

	Number of symptomatic plants per replicate plot				Total	Overall %
Border Row Type	1	2	3	4	Symptomatic Plants	Symptomatic Plants
Treatment A:						
Susceptible borders	4	1	3	3	11 of 24	46%
Treatment B:						
Resistant borders	5	3	1	1	10 of 24	42%

Conclusions

The use of PRSV-resistant papaya in border rows did not prevent transmission of the virus to the susceptible cultivar 'Red Lady'. There are numerous reasons why a border row management strategy may have been successful in Hawai'i but not in our experience. These include the presence of an older planting of PRSV-infected papaya field within 50 ft of our trial, frequent high wind conditions in Homestead (which may disperse the aphid vector widely), and the early stage at which we transplanted seedlings to the field.

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University of Florida/IFAS, Tropical Research and Education Center—Efforts to Register Pest Control Substances for Florida's Tropical Fruit Industry, 1999–2019

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Insect and disease pests are a major financial constraint for Florida's tropical fruit industry. Every year one or more invasive insect, disease, or weed becomes established in Florida resulting in mild to drastic reductions in fruit production and/or quality. The IR-4 Project was established in 1963 to facilitate registration of conventional and biopesticides for specialty crops in the U.S. These specialty crops make up about 40% of the U.S. crop value. Florida's tropical fruit industry consists of about 14,562 acres and is worth more than $300 million annually. The IR-4 Project is a collaboration among USDA (NIFA, ARS, APHIS, and FAS), Land Grant Universities (State Agricultural Experiment Stations), EPA, the crop protection industry, and agricultural crop producers. The program is designed to determine which crop protection substances are efficacious, then conduct field trials to collect fruit samples for residue analysis, and ultimately register crop protection substances with the EPA for use by crop producers. An IR-4 Field Station was established at the Tropical Research and Education Center (TREC) in Homestead, FL, in 1993. Since that time researchers have conducted four to twenty field trials per year. This collaboration with IR-4 has resulted in the registration of over 52 pest control active ingredients and numerous brand-named products for Florida's tropical fruit producers since 1999.

Florida's tropical and subtropical fruit industry has had a long and significant social and economic impact locally, nationally and internationally (Crane, 2018; Degner et al., 2002a; Degner et al., 2002b; Krome and Goldweber, 1987; Campbell, 1971; Goldweber, 1961; Knight et al., 1984; Steele and Crane, 2006). Horticultural scientists, producers, and fruit enthusiasts have introduced hundreds of fruit crop species and cultivars since the 1890's (Krome and Goldweber, 1987; Fairchild, 1934; Fairchild 1937; Fairchild, 1938; Imbruce, 2007; McGuire et al., 1999; Whitman and Biebel, 1962). Today in Florida there are an estimated 14,562 acres of tropical fruit crops distributed in 16 counties (Crane, 2018). There is no current estimate of the economic impact of Florida's tropical fruit industry; however, earlier estimates and estimates of individual crops suggest the overall economic impact exceeds $300 million annually. Fruit crops include avocado, banana, caimito, canistel, carambola, dragon fruit, guava, guanabana (soursop), jackfruit, longan, lychee, mamey sapote, mango, miracle fruit, papaya, passion fruit, sapodilla, *Spondias* sp., sugar apple, and wax jambu (Crane, 2018).

The challenges faced by tropical fruit producers from insects, diseases, and weeds have been devastating economically, socially, and environmentally. Two recent Florida examples include citrus greening (Alvarez et al, 2016; Singerman and Rogers, 2020) and laurel wilt of avocado (Mosquera et al., 2015). Both have resulted in dramatic decreases in the number of farms producing citrus and avocado, reduced production, and the dislocation of ancillary industries (e.g., juice plants). International trade and travel have exacerbated and intensified the pest problems faced by agriculture (Jenkins, 1996; Penca et al., 2016; Work et al., 2004).

Historically, few conventional pest control products were available to commercial tropical fruit producers in the U.S. and Florida in particular. This is because the potential sales value of pest control products is inadequate to meet the costs of testing and registering them with the Environmental Protection Agency (EPA) which are generally borne by the manufacturers. Fortunately, the need for pest control products for use on minor crops was recognized, and in 1963 the Directors of the State Agricultural Experiment Stations (Land Grant Universities) established a collaborative program designated by the USDA as the IR-4 Project (IR-4, 2020a). This collaboration includes the USDA-National Institute of Food and Agriculture, USDA-Agricultural Research Service, USDA-Foreign Agricultural Service, USDA-Animal and Plant Health Inspection Service, State Agricultural Experiment Stations (Land Grant Institutions), the US-Environmental Protection Agency, the agrochemical industry, commodity groups and growers. The mission of the project is to facilitate regulatory (EPA) approval of sustainable pest management products for specialty crops and specialty uses to promote the public wellbeing (IR-4, 2020a).

Before the establishment of recent crop groupings for tropical and subtropical fruits, each tropical or subtropical fruit crop and active ingredient (ai) had to be field tested and residues deter-

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Table 1. EPA crop groups and relevant commercial subtropical and tropical fruit crops included from Florida. (IR-4, 2020b).

Crop group	Subtropical and tropical fruit crops grown commercially in Florida
23B-Tropical and Subtropical, Medium to Large Fruit, Edible Peel	ambarella, feijoa, fig, cattley guava, guava, jaboticaba, Indian jujube, mombin, tamarind
24A-Tropical and Subtropical, Small Fruit, Inedible Peel	bael fruit, longan, lychee, Spanish lime, wampi
24B- Tropical and Subtropical, Medium to Large Fruit, Inedible Peel	abiu, avocado, banana, black sapote, canistel, green sapote, mango, papaya, persimmon, pomegranate, star apple, white sapote
24C-Tropical and Subtropical, medium to large, Rough or Hairy, Inedible Peel	atemoya, custard apple, ilama, jackfruit, mamey-apple, pineapple, rambutan, sapodilla, mamey sapote, soursop, sugar apple
24D-Tropical and Subtropical Vine, Inedible Peel	dragon fruit (pitahaya, pitaya), prickly pear (tuna)
24E-Tropical and Subtropical Vine, Inedible Peel	giant granadilla, monstera, passionfruit (banana, purple, yellow and hybrids)

mined for each crop/ai combination (EPA, 2016). This greatly increased the workload necessary to obtain pesticide registrations for the hundreds of "minor" crops grown in the U.S. For example, prior to crop grouping, registering a fungicide (FX) for lychee and longan meant conducting independent field trials with both lychee and longan. With crop groupings, registering fungicide FX only requires field residue trials on lychee since data from lychee also covers longan. To address this workload issue, the IR-4 Project collaborators established tropical and subtropical crop groups in 2016 which have since been updated and modified (Table 1). Crop groups are formed around the potential ai residue levels remaining on produce after pesticide applications and are based on fruit size, edible versus non-edible peel, and peel surface characteristics (e.g., smooth, bumpy). Each crop group is represented by one or more "test" crops which then represent all the other members of the crop group. Active ingredient residue trials are conducted on the test crop and the results apply to all or most of the crops within the crop grouping. In general, this has reduced the number of field trials necessary to register an ai for multiple crops within a crop group and has streamlined the ai registration process for minor crops. However, in most cases for an ai to be registered for use the ai/test crop combinations must be tested at several IR-4 Field Stations which represent the different environmental conditions under which the ai would be used. For tropical and subtropical fruit crops that could include IR-4 Stations in what is called the Tropical Region i.e., Florida, California, Puerto Rico, and Hawaii.

During 1988, the senior author began to participate in the IR-4 Program by conducting field trials performed to collect fruit samples for residue testing (Crane et al., 1998). In 1993 an IR-4 field Station was established at the University of Florida/IFAS Tropical Research and Education Center in Homestead, FL. Over an eleven year (1988–98) period, 87 trials were conducted which resulted in registration of a total of 10 new pest control active ingredients for avocado (1), mango (3), guava (1), papaya (3), and passion fruit (2). From 1993 to the present, there have been four Field Research Biologists (FRB) working with the first author (Robert Sanford, Osvany Rodriguez, Reed Olszack and Rebecca Tannenbaum). These FRB's executed the pest control field trials necessary to collect the fruit samples which were analyzed to determine ai residue levels that were then used in petitions to register active ingredients with the Environmental Protection Agency (EPA).

Registration of these ai's involves a vetting process that included the consensus of all the IR-4 collaborators on which ai/crop trials to execute. Once a project is approved, IR-4 Project staff develop protocols and FRB's execute the protocols in the field. Field trials involve not only ai applications to test crops but quality assurance unit (QA) inspections of the trial to be sure trials have complied with Good Laboratory Practices. Produce samples from field trials are sent to laboratories for residue analysis. IR-4 Headquarter staff then compile and write petitions to EPA for the registration of the ai for the crop (crop group). This process, from approval for a residue trial to full registration of an ai for

Table 2. Crop group, test crop, and active ingredients field tested 1999–2019 by the TREC IR-4 Station varied by crop.

Crop group	Test crop used	Active ingredient field tested
23B	Guava	abamectin, acequinocyl, azoxystrobin, azoxystrobin-difenoconazole, bifenazate, buprofezin, chlorothalonil, clofentezine, cyrodinil+fludioxonil, difenoconazole, diquat, fenpropathrin, fluopyram-tebuconazole, flumioxazin, glufosinate, imidacloprid, lambda-cyhalothrin, mancozeb, methoxyfenozide, propamocarb-HCL, pryriproxyfen, spirodiclofen
24A	Lychee (longan)	abamectin, acequinocyl, azoxystrobin, bifenazate, buprofezin, cyrpodinil+fludioxonil, imidacloprid, methoxyfenozide, pyriproxyfen, tebuconazole
24B	Avocado (banana only*, mango only**, papaya only***)	abamectin, acequinocyl, acetamiprid, azoxystrobin, azoxystrobin-fludioxonil, bifenazate***, buprofezin, chlorothalonil**/***, clofentezine, cyprodinil+fludioononil, difenoconazole***, diquat, diuron***, etoxazole, fenpropathrin, fenproximate, flumioxazin, folpet, glufosinate, imidacloprid, lambda-cyhalothrin-thiamethoxam, mefenoxam***, methoxyfenozide***, methidathion**, milbemectin, NAA, novaluron, oxythiapiprolin, propiconazole, propiconazole-fludioxonil, spirotetramat*, thiamethoxam, tebuconazole, tolfenpyrad, trifloxystrobin***, triflumizole***, zeta-cypermethrin
24C	Sugar apple (atemoya)	bifenazate, imidacloprid, mancozeb, mefenoxam, pyriproxyfen
24E	Passionfruit	Imidacloprid

Table 3. Crop group, test crop, and active ingredient registered over the period of 1999-2019.

Crop group	Test crop used	Number of new active ingredients registered	Active ingredient registered
23B-Tropical and Subtropical, Medium to Large Fruit, Edible Peel	Guava (*Psidium guajava*)	16	*Insecticides:* abamectin, acequinocyl, clofentezine, bifenazate, buprofezin, fenpropathtrin, imidacloprid, methoxyfenozide, pryriproxyfen, spinosad *Fungicides*: azoxystrobin, difenoconazole, cyrpodinil+fludioxonil, fludioxonil *Herbicides:* carfentrazone-ethyl, paraquat
24A-Tropical and Subtropical, Small Fruit, Inedible Peel	Lychee (*Litichi chinensis*); sometimes longan (*Dimocarpus longan*)	13	*Insecticides:* abamectin, acequinocyl, bifenazate, buprofezin, fenpropathtrin, imidacloprid, methoxyfenozide, pyriproxyfen *Fungicides:* azoxystrobin, cyrpodinil+fludioxonil, difenoconazole, fludioxonil, tebuconazole
24B-Tropical and Subtropical, Medium to Large Fruit, Inedible Peel[z]	Avocado (*Persea americana*)	27	*Insecticides:* abamectin, acequinocyl, acetamiprid, bifenazate, buprofezin, chlorantraniliprole, clofentezine, etoxazole, fenpropathrin, fenproximate, fludioxonil, imidacloprid, novaluron, pyriproxyfen, spinetoram, spinosad, sprodiclofen, thiamethoxam, tolfenpyrad, zeta-cypermethrin *Fungicides:* azoxystrobin, cyprodinil+fludioxonil, folpet *Herbicides:* carfentrazone-ethyl, paraquat *Plant growth regulators:* gibberellic acid, NAA
	Banana (*Musa* spp.)	6	*Insecticides:* fenpropathrin, imidacloprid, spinetoram, spinosad, spirotetramat *Fungicide:* mancozeb
	Mango (*Mangifera indica*)	7	*Insecticides:* etoxazole, imidacloprid, methidathion, methoxyfenozide *Fungicide:* chlorothalonil, mancozeb *Herbicide:* glyphosate
	Papaya (*Carica papaya*)	9	*Insecticides:* methoxyfenozide *Fungicides:* difenoconazole, mancozeb, mefenoxam, myclobutanil, trifloxystrobin, triflumizole *Herbicide:* diuron, oryzalin
24C-Tropical and Subtropical, medium to large, Rough or Hairy, Inedible Peel	Sugar apple (*Annon squamosa*); sometimes atemoya (*A. cherimola* × *A. squamosa*)	12	*Insecticides:* bifenazate, buprofezin, imidacloprid, pyriproxyfen, spinosad, methoxyfenozide *Fungicides:* azoxystrobin, fludioxonil, mancozeb, mefenoxam *Herbicides:* carfentrazone-ethyl, paraquat
24D-Tropical and Subtropical Cactus, Inedible Peel	Dragonfruit (*Hylocereus undatus*)	3	*Fungicides:* azoxystrobin, cyprodinil+fludioxonil, fludioxonil
24E-Tropical and Subtropical Vine, Inedible Peel	Passionfruit (*Passiflora edulis* and hybrids)	10	*Insecticides:* abamectin, buprofezin, fenpropathrin, imidacloprid, methoxyfenozide, pyriproxyfen, spinosad *Fungicides:* azoxystrobin, chlorothalonil, cyprodinil+fludioxonil

[z]Some ai registered on avocado are also registered on banana and mango. Others are specific to banana and mango.

a crop (crop group) takes three to seven years. However, not all ai registrations required field trial/residue testing since data from previous or alternative crops or other tropical regions can sometimes bridge the data gaps.

Herein we report on the crops and pesticide active ingredients tested (Table 2) and those successfully registered through the efforts of the TREC IR-4 Station participation in the IR-4 Project from 1999 to 2019 (Table 3). The number of new ai's registered ranged from three for crop group 24D to 27 for crop group 24B (Table 4).

From 1999 to 2019, the TREC IR-4 Field Station conducted 269 field residue trials with an average of 13 trials performed each year (range four to 19). The number of ai's tested by the TREC IR-4 Field Station ranged from one for Crop Group 24E (passionfruit) to 26 for Crop Group 24B (Table 2). A total of 52 active ingredients were tested from 1999 to 2019. The number of ai tested in the field per year ranged from two to eight and the number of crops used from three to eight. The number of independent field trials required for each ai and test crop combination ranged from one to four. Field trials were performed at TREC and on the farms of many local tropical fruit growers.

The TREC IR-4 Field Station's participation with the IR-4 Project has been important to the development of Florida's tropical fruit industry because it has resulted in the EPA registration of numerous needed pest control products for producers. Without the IR-4 Project, the pest problems that occur on many subtropical and tropical fruit crops would make economically viable production of many of these crops tenuous at best. The TREC IR-4

Table 4. Crop group, test crop, and active ingredients field tested 1999-2019 by the TREC IR-4 Station varied by crop.

Crop group	Test crop used	Active ingredient field tested
23B	Guava	abamectin, acequinocyl, azoxystrobin, azoxystrobin-difenoconazole, bifenazate, buprofezin, chlorothalonil, clofentezine, cyrodinil+fludioxonil, difenoconazole, diquat, fenpropathrin, fluopyram-tebuconazole, flumioxazin, glufosinate, imidacloprid, lambda-cyhalothrin, mancozeb, methoxyfenozide, propamocarb-HCL, pryriproxyfen, spirodiclofen
24A	Lychee (longan)	abamectin, acequinocyl, azoxystrobin, bifenazate, buprofezin, cyrpodinil+fludioxonil, imidacloprid, methoxyfenozide, pyriproxyfen, tebuconazole
24B	Avocado (banana only*, mango only**, papaya only***)	abamectin, acequinocyl, acetamiprid, azoxystrobin, azoxystrobin-fludioxonil, bifenazate***, buprofezin, chlorothalonil**/***, clofentezine, cyprodinil+fludioononil, difenoconazole***, diquat, diuron***, etoxazole, fenpropathrin, fenproximate, flumioxazin, folpet, glufosinate, imidacloprid, lambda-cyhalothrin-thiamethoxam, mefenoxam***, methoxyfenozide***, methidathion**, milbemectin, NAA, novaluron, oxythiapiprolin, propiconazole, propiconazole-fludioxonil, spirotetramat*, thiamethoxam, tebuconazole, tolfenpyrad, trifloxystrobin***, triflumizole***, zeta-cypermethrin
24C	Sugar apple (atemoya)	bifenazate, imidacloprid, mancozeb, mefenoxam, pyriproxyfen
24E	Passionfruit	imidacloprid

Field Station will continue to participate in the highly successful IR-4 Project which serves the multi-billion-dollar specialty crop industries in the U.S.

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O47-16-10: A Pierce's Disease-resistant and Premium Wine Producing Bunch Grape Hybrid with Later Bud Burst in Spring

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Additional index words. hot-humid environment, adaptation, frost damage, productivity, fruit rot, vine vigor

'O47-16-10' is a newly developed breeding line at the Viticulture Center, Florida A&M University, Tallahassee, Fl. This premium wine producing bunch grape is superior in being adaptable to Florida's hot-humid growing environment and is resistant to Pierce's Disease. It posses several additional advantages preferred by grape industry, such as self-fertile flowers, high productivity, and low ripe rot during ripening. The later starting of vegetative activity of the vine in spring could minimize the frequent later frost damage for the grape industry in Florida.

Florida grape industry is challenged all the time by Pierce's disease (PD) due to the hot-humid growing environment, which limits growing of *Vitis vinifera* grapes that produce exceptional quality wines to virtually zero in the region. The commercial growing of bunch grapes has solely been based on a few Florida hybrid grapes that are adapted to Florida's hot-humid climate and resistant or tolerant to PD, such as 'Blanc du Bois', 'Stover', and 'Conquistador'. These cultivars could produce good wines, but production is costly due to their susceptibility to other diseases related to heat and humidity such as the fungal disease anthracnose and downy mildew, which require intense spray programs. The ripe rot on 'Blanc du Bois' during ripening might also be a severe problem for both growers and wineries.

Florida grape industry frequently suffers from spring frost damage in addition to the heat and humidity related diseases. These often result in significant production losses or even economic disasters. Open buds and young shoots are sensitive to freezing temperatures, so a short period of exposure to freezing during a spring frost could be lethal to open primary buds and shoots. After severe damage or the death of primary shoots, the subsequent development of shoots from the secondary growing points or buds produce some fruit, but this could be 50% lower than production from primary shoots for most grapes.

With these problems facing the local grape industry, focused continuous breeding efforts to improve existing cultivars and develop new grape cultivars with superior qualities is needed for the development and growth of the grape and wine enterprises, integrating PD resistance, adaption to Florida's hot-humid climate, and fine wine quality. All these would allow a wine grape to be grown successfully in Florida. The grape breeding program in Florida A&M University (FAMU) has been working intensively for the past 2 decades to develop fine wine producing grapes that can grow successfully in Florida. This work has resulted in several valuable hybrids, among which 'O47-16-10' has showed good potential as a premium wine producing selection, and is reported on here.

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Origin

'O47-16-10' originated from a cross between 'N18-6' and 'Merlot' in 1999. The female parent 'N18-6' is a complex germplasm line, which has shown a high degree of resistance to PD and anthracnose over the past 20 more years. It also possesses several desirable commercial characteristics such as high yield, high sugar level, moderate vigor, and easy canopy management. It also shows strong tendency to transmit these characters to its decedents. This selection has been used extensively in our breeding programs (Ren, et al. 2009). 'Merlot', the male parent, is a leading premium wine grape cultivar that produces both varietal and blending red wines. 'Merlot' has been less bothered by diseases than other *V. vinifera* cultivars that are PD free and cage-grown in Tallahassee, FL.

The hybrids were planted in Summer 2000, but the majority of them died before 2008 with only a few vines surviving in July, 2020; 'O47-16-10' is one of the survivors. It has been growing well since 2000, has shown a superior adaptability to Florida's environment and has PD resistance. The vine's horticultural potential first received attention in 2005 while its enology traits have been observed since 2007. Both horticultural and enological characteristics have been evaluated and compared with 'Blanc du Bois' and 'Stover', the two most important Florida grown wine cultivars, under same conditions.

Plant and Wine Characteristics

Plant characteristics

Flowers and Inflorescences. 'O47-16-10' produces hermaphrodite (self-fertile, perfect) flowers, as both male and female organs of 'O47-16-10' flowers are fully developed, pollinators are not necessarily required. No cap-sticking which has ever been observed during evaluation indicates that the flowers of the hybrid are healthy. The flower clusters generally grow at 2nd to 4th node (Fig. 1).

Productivity. 'O47-16-10' is highly productive (Fig. 2). A 10-ft canopy of 'O47-16-10' which was vine trained as a 2-arm cordon on a single wire system with spur pruning produced on

Fig. 1. Young shoots and inflorescences of 'Blanc du Bois' (top), 'Stover' (middle), and 'O47-16-10' (bottom). The differences in shoot sizes corresponds with vine vigor.

Fig. 2. Fruit clusters and productivity of 'O47-16-10'.

average 18 lb grapes, which was the same as that of the high yield cultivar 'Blanc du Bois', and was more than 'Stover' (Table 1). These yields have been constant during the evaluation period.

The spur productivity of 'O47-16-10' was 417 g fruit/spur, which was similar to the high yield cultivar 'Blanc du Bois', but and greater than 'Stover' (Table 1). As the basic productive part of a grape vine, spur productivity is useful in grape yield studies. It has suggested the high production potential of 'O47-16-10'.

Fruit clusters. Mid-large semi-dense fruit clusters weigh roughly 125 g, consist of about 87 individual fruits, the pedicels are partial visible (Figs. 2 and 3). Fruits are movable and it is somewhat difficult to detach fruits from the pedicels. The semi-dense clusters enable air flow and sprays penetrating inside the clusters. These may reduce fruit rot during ripening and is a trait desired by grape growers.

Fruit profile of 'O47-16-10'. The fruits of 'O47-16-10' are small and round in shape generally, about 1.3cm in diameter with the length slightly longer than the diameter, and weigh 1.4g (Table 1). Fruit skin is medium thin and smooth with a dark green-yellow color (Fig. 3) and can easily be separated from the pulp. Fruits are semi-soft textured, with neutral or no outstanding flavors.

Fruit sugar content (SSC) averaged 16.2%, titratable acid averaged 0.70g/L with pH 2.98 (Table 1).

Adaptation to hot-humid environment and disease resistance

'O47-16-10' has been growing and fruiting well in Tallahassee, Fla since 2000. During this time, plants have experienced numerous hot-humid related pressures and have shown an outstanding adaptation to Florida's hot-humid growing environment.

Using a 0-5 score criteria, 'O47-16-10' has not shown PD symptoms while light PD symptoms could be observed occasionally on 'Blanc du Bois' and 'Stover (Table 2).

'O47-16-10' showed minor anthracnose symptoms which was lighter than in 'Blanc du Bois' during evaluation period (Table 2).

'O47-16-10' had less fruit rot or ripe rot at harvest. This is a frustrating epidemic for Florida's grape industry. Its 4% fruit rot rate was similar to that of 'Stover', but lower than 'Blanc du Bois' (Table 1). These should make difference for the grape industry; a lower fruit rot rate suggests that growers may be able to harvest more fruit for commercial use while a higher fruit rot rate suggests the opposite.

Vine vigor

The vines of 'O47-16-10' grow vigorously to very vigorously, i.e., the growth of a shoot could easily reach 10 to 20 feet in a growing season. Vine have largr internodes and leaves (Fig. 1, Table 2), which results in a higher pruning weight. The average pruning weight was 5.6 lb/vine from 10-feet of canopy. 'O47-16–10' vines are trained as a 2-arm cordon single wire system with spur pruning techniques, which was the same as the vigorous 'Blanc du Bois', and was higher than 'Stover' (Table 2). The internode size of 'O47-16-10' averaged 11.0cm in length and 1.11cm in diameter, which were larger than the vigorous 'Blanc du Bois' and the moderately vigorous 'Stover' (Table 2). Similarly, 'O47-16-10' has larger leaves (12.8 × 14.9cm, L×W) than either 'Blanc du Bois' or 'Stover' (Table 2). Because of its high vine vigor, 'O47-16-10' would not require grafting, which would substantially reduce planting costs.

Later bud break and blooming—Annual growth cycle of the grape vine

The start of the vegetative activities of 'O47-16-10' in the spring is about 3 weeks later than almost all bunch grapes including 'Blanc du Bois' and 'Stover' in Tallahassee, FL (Table 2), which enabled 'O47-16-10' to avoid late frost damage during the evaluation period, while other cultivars frequently suffered

Table 1. Productivity, fruit enological traits, and wine evaluation of 'O47-16-10' compared with two premium Florida wine hybrids

Cultivar	Spur fruit wt (g)	Yield (lb/vine)	Fruit rot rate (%)	Fruit size (g)	SSC (%)	pH	TA (g/L)	Wine score[z]
'O47-16-10'	417	18	4	1.4	16.2	2.98	0.70	13.0
'Blanc du Bois'	428	19	37	2.8	17.9	3.05	0.57	--
'Stover'	227	13	4	2.4	15.2	3.08	0.51	12.6

[z]American Wine Society (AWS) evaluation standard, total of 20 scores.

SSC = soluable solids content; TA = tritatable acidity.

Fig. 3. Typical cluster and fruit color of 'O47-16-10'.

severe loses (Fig. 4). This late blooming trait would have high potential for grape production areas with spring frost problems.

Wine Characteristics

'O47-16-10' produced a delicious white wine with a clean pale-yellow color, neutral pleasant flavor, clear finish, and excellent stability. The wine score was determined by a taste panel according to the American Wine Society (AWS) evaluation procedure, in which the total 20 scores were divided into: appearance 0-3; aroma and bouquet 0–6; taste and texture 0-6; aftertaste 0–3; and overall impression 0–2. The 13.0 point of the 'O47-16-10' wine evaluation was similar to the 12.6 points of the fine wine made from 'Stover' (Table 1), which means that 'O47-16-10' can produce fine wine. The wine styles of 'O47-16-10' vary from dry to semi-dry; it can be used to produce varietal wine and blending wine.

The major advantages of the 'O47-16-10' as a fine wine producing grape are its superior adaptability to Florida's hot-humid environment, resistance to PD, satisfactory productivity, and ability to produce premium wine. The selection could further benefit the grape industry by having minimal late frost damage during bud burst and blooming in spring.

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Fig. 4. Developmental stages and frost damage to 'O47-16-10' (top) and 'Stover' (bottom) on 3/16/2017.

Table 2. Horticultural characteristics of 'O47-16-10' and two premium Florida wine hybrids.

Cultivar	Vine vigor	Pruning wt (lb/vine)	Internode (L × D, cm)	Leaf size (L × W, cm)	PD symptom (0~5)	Anthracnose symptom (0~5)	Bud break[z]	Blooming[z]	Fruit ripening
'O47-16-10'	vigorous	5.6	11.0 × 1.11	12.8 × 14.9	none	1	mid Mar.	late Apr.-mid May	early-mid Aug.
'Blanc du Bois'	vigorous	5.2	8.0 × 1.05	9.1 × 12.2	occasional	3	end Feb.	early-mid Apr	mid July
'Stover'	moderate	2.6	5.4 × 0.82	6.8 × 8.5	occasional	2	end Feb.	early-mid Apr.	mid-late July

[z]Biological stages developed in Tallahassee, FL.
PD = Pierce's desease.

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Low Cost High Tunnel versus Open Field Production of Organically Grown Strawberries in North Florida: A Three-year Evaluation

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In north Florida, crop production can be limited by freezing temperatures during winter months and as such, many fresh market, organic and small scale producers utilize season extension techniques to protect specialty crops from damage. Low cost high tunnels (LCHT) are temporary crop protective structures that can be easily constructed in the field to protect crops from adverse weather conditions and pests. They are naturally heated and ventilated by roll-up sides as a means of controlling environmental conditions inside the tunnel. In this study, we present the results of a 3-year evaluation of strawberries grown in a LCHT versus open field (OF) treatment. The objective was to determine whether the LCHT provided any agronomic benefits, relative to fruit yield, over the OF. Four strawberry (*Fragaria ×ananassa*) cultivars [Sensation™, ('Florida 127'), 'Florida Brilliance', 'Florida Radiance', and 'Florida Beauty'] were grown in each production system. Experimental plots consisted of 16 subplots each containing 16 strawberry plants per cultivar in a randomized complete block design with four replications. Yield data were collected weekly during the 2016/17, 2017/18, and 2019/20 Fall through Spring growing seasons. Descriptive statistics were generated using SAS ver. 9.4. The three-year average yield from the LCHT and OF treatments were 1744 lb/acre and 949 lb/acre, respectively. An independent samples *t* test was used to compare treatment means. The yield obtained from the LCHT was significantly higher ($P < 0.001$). The study concluded that the LCHT is a sustainable alternative to OF production of strawberries in north Florida.

For the past four winter seasons in north Florida (excluding the 2018-19 strawberry season due to damaged caused by Hurricane Michael), low cost high tunnel (LCHT) production of organically grown strawberries has been evaluated against the risk of winter injury or damage to strawberry plants, flowers, and fruit. This damage can occur when ambient air temperatures fall below 32 °F (0 °C). To determine whether a LCHT can provide any agronomic benefits, including fruit yield, strawberry production inside the LCHT were compared to fruit production in an open field (OF). This comparative study also served as an extension outreach demonstration to validate the feasibility of growing strawberries for small-scale production.

In the first year of the study, Oct. 2016 to Apr. 2017, it was determined that the risk of winter injury or damage could reduce marketable fruit yield thereby reducing crop profitability (Bolques et al., 2018). The study was repeated for a second year during the Oct. 2017 to Apr. 2018 strawberry season. In this paper, we discuss the results of a low cost high tunnel structure versus open field production of organically grown strawberries in North Florida during the third and final year.

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Materials and Methods

Experimental Design. This study was conducted at the Florida A&M University, Research and Extension Center in Quincy, FL, and covers three strawberry growing seasons. For the first season, Oct. 2016 to Apr. 2017, four strawberry cultivars [Sensation™ (Florida 127) (Whitaker et al, 2014), Winterstar™ (FL 05-107) (Whitaker et al., 2012), 'Florida Radiance', and 'Strawberry Festival'] were evaluated for their performance in two 12 ft (3.6 m) wide × 100 ft (30 m) low cost high tunnels and one 12 ft (3.6 m) wide × 100 ft (30 m) open field arrangements. Likewise, for the 2017–2018 growing season, LCHT and OF strawberry cultivars were the same with the exception of 'Strawberry Festival' which was replaced by 'Florida Beauty'. For the final year, 2019–2020, LCHT and OF strawberry cultivars were the same with the exception of Winterstar™, which was replaced by 'Florida Brilliance'. The objectives, methods and data analytical procedures were the same as those utilized in years 1 and year 2 studies (Bolques et al., 2018 and Bolques et al., 2019).

The soil at the study site is an Orangeburg loamy sand with moderate permeability and water holding capacity (USDA NRCS, 2017). Soil preparation consisted of growing a sunn hemp cover crop, *Crotalaria juncea*, during the late summer (Aug.–Sept.). The cover crop was selected for its ability to fix nitrogen and when incorporated into the soil, can release allelopathic compounds

toxic to plant parasitic nematodes (Wang, 2008). Following cover crop incorporation, 5 lb (2.3 kg) of preplant fertilizer, Nature Safe 8–3–5 Super Fine organic fertilizer, incorporated into the soil for each production bed prior to forming the plastic mulch raised beds, which also included two 5/8 inch (1.6 cm) T-Tape drip irrigation lines per bed. Two rows were designated for the OF treatment and four for the LCHT. Commercially available strawberry plant plugs were used for the evaluations and were planted using a randomized complete block design with 4 replications. Each treatment consisted of four 50-ft (15 m) subplots. Each subplot contained four strawberry cultivars with 16 plants per cultivar. The planting arrangement throughout the experimental plots consisted of double rows spaced 18 inches (45) cm × 18 inches (45) cm apart. At the end of each experimental plot, four strawberry plugs were planted as end of row buffers.

The crop was irrigated daily for an hour the first week, then three times per week or as needed. At mid-season (January), individual plants were top dressed with a teaspoon of Nature Safe 8–3–5 Super Fine organic fertilizer every three weeks. The crop was scouted weekly and applied control measures were recorded. Ripe (red) fruits were sampled weekly from eight plants in each experimental subplot. Fruit yield and marketable yield were measured by weight.

Low cost high tunnel. The LCHT was constructed based on design specifications by Coolong (2012) with modifications to the structure anchoring (Bolques et al., 2016). Otherwise, LCHT construction was the same as Bolques et al. (2018 and 2019). A "how to video" file on how to construct a LCHT is available on YouTube (Bolques, 2017). Only 1 LCHT was evaluated during the third year which included structural improvements to the hoop assembly, anchor system, and rope tie down.

Hoop structural improvements. The major structural component of the LCHT was the use of 1 in (2.5 cm) in diameter by 20 ft (6 m) long polyvinyl chloride (PVC) pipe to form a hoop segment. The 1 in × 20 ft PVC pipe used to make a hoop section was replaced by two galvanized steel chain link fence top rails, each measuring 1 3/8 in (42 cm) × 10.5 ft (3.2 m). A DY12-B hoop bender (Lost Greek Greenhouses, LLC, Mineola, TX), was used to bend the metal pipes. Two bent metal hoop sections inserted at the belled end and held together by a Tek screw, completed a metal hoop section.

Anchor structural improvements. Upgrading from PVC to metal hoops also meant upgrading the anchoring system. As a result of the belled end design of the metal hoop pipe, 2 different anchors was needed for each metal hoop section: a 2 ft, 1 3/8-in galvanized steel chain link fence top rail and a 2 ft, 11-in metallic electrical conduit. Both were installed 18 in. into the ground leaving 6 in exposed above ground. The belled end of the metal hoop was installed into the 1 3/8-in anchor and the non-belled end was installed over the 1-in anchor.

Rope assembly improvements. A braided nylon rope was used to hold the tunnel poly plastic in place over the hoop sections. At the metal hoop and anchor insert points, a 3/4-in, two-hole metal strap was mounted using 5/16 hex screws making a strong joint between the hoop and the anchor.

Results and Discussions

The results from the three-year evaluation are shown in Tables 1, 2, and 3, respectively. In each table the treatment means compared are the mean yields of the cultivars within each production

Table 1. Mean yield (lb/acre) of four organically grown strawberry cultivars from open field and low cost high tunnel production, Fall 2016–Spring 2017 in north Florida.

Treatment	Cultivar	Min	Max	Mean[z]	±SD
Open field	Strawberry Festival	0.00	5101.4	1326.4 a	1333.81
	Florida Radiance	0.00	3464.3	524.1 b	551.1
	Winterstar™	0.00	6463.1	1682.2 a	1617.1
	Sensation	0.00	6099.6	1361.8 a	1391.8
Low cost high tunnel	Strawberry Festival	232.7	8623.1	3426.1 a	2142.4
	Florida Radiance	67.3	6151.1	1640.2 c	1226.6
	Winterstar™	0.00	7013.7	2598.5 b	1750.3
	Sensation	0.00	10164.1	2835.1 b	1623.8

[z]Means within each production system with the same letter are not significantly different at $\alpha = 0.05$.

Table 2. Mean yield (lb/acre) of four organically grown strawberry cultivars from open field and low cost high tunnel production, Fall 2017–Spring 2018 in North Florida.

Treatment	Cultivar	Min	Max	Mean[z]	±SD
Open field	Florida Beauty	0.00	3575.2	776.9 b	957.0
	Florida Radiance	0.00	9267.8	1810.3 a	1993.5
	Winterstar™	0.00	4669.6	1108.9 b	1139.9
	Sensation	0.00	5400.4	1224.2 ab	1419.61
Low cost high tunnel	Florida Beauty	0.00	4888.4	922.3 b	788.0
	Florida Radiance	57.4	4866.6	1504.7 a	804.7
	Winterstar™	107.0	4628.0	1437.7 a	996.3
	Sensation	0.00	4595.3	1232.8 a	919.6

[z]Means within each production system with the same letter are not significantly different at $\alpha = 0.05$.

Table 3. Mean yield (lb/ac) of four organically grown strawberry cultivars from open field and low cost high tunnel production, Fall 2019–Spring 2020 in north Florida.

Treatment	Cultivar	Min	Max	Mean[z]	±SD
Open field	Florida Beauty	0.00	3179.1	477.8 a	680.6
	Florida Radiance	0.00	3426.7	606.8 a	794.9
	Florida Brilliance	0.00	2456.1	456.6 a	555.0
	Sensation	0.00	3575.2	543.7 a	682.7
Low cost high tunnel	Florida Beauty	0.00	4318.0	812.4 a	957.14
	Florida Radiance	0.00	6199.7	1565.9 ab	1473.5
	Florida Brilliance	0.00	6784.0	1290.1 ab	1309.1
	Sensation	0.00	5357.9	1232.2 b	1165.8

[z]Means within each production system with the same letter are not significantly different at $\alpha = 0.05$.

Fig. 1. Mean yield (lb/acre) of four organically grown strawberry cultivars in north Florida by season (Oct.–Apr.) in a low cost high tunnel versus open field (**A–C**) and overall strawberry production for all three seasons, excluding the 2018–19 season (**D**).

system. The yield (lb/acre) comparisons of the two production systems (OF vs. LCHT) are depicted in Fig. 1. This comparison allows for a visual determination of whether the LCHT provided any yield advantages over the OF (control).

Evaluation of strawberry cultivars in the OF and LCHT (2016–17). In the OF, mean yield ranged from 524 lb/acre (587 kg/ha) for 'Florida Radiance' to 1682 lb/acre (1885 kg/ha) for Florida brilliance. For the 2016 growing season, 'Florida Radiance' produced significantly lower yield compared to the other 3 cultivars (Table 1). Winterstar™ was notably the highest producer in the OF. However, when compared to 'Strawberry Festival' and 'Sensation™', its yield was not significantly higher than these two cultivars (Table 1).

In the LCHT, mean yield ranged from 1640 lb/acre (1838 kg/ha) for 'Florida Radiance' to 3426 lb/acre (3840 kg/ha) for 'Strawberry Festival'. The yield from 'Florida Radiance' was significantly lower compared to the other 3 cultivars (Table 1). 'Strawberry Festival' produced significantly higher yield compared to the other cultivars in the LCHT. There were no significant differences between the yield from Winterstar™ and Sensation™.

Evaluation of strawberry cultivars in the OF and LCHT (2017–18). The mean yield in the OF ranged from 777 lb/acre (871 kg/ha) for 'Florida Beauty' to 1810 lb/acre (2029 kg/ha) for 'Florida Radiance'. Mean yield from 'Florida Radiance' in the OF was significantly higher than for the other three cultivars. The yields for 'Florida Beauty', Winterstar™, and Sensation™, were not significantly different (Table 2).

In the LCHT, mean yield ranged from 922 lb/acre (1033 kg/ha) ('Florida Beauty') to 1504 lb/acre (1686 kg/ha) ('Florida Radiance'). The yield obtained from 'Florida Beauty' in the LCHT was significantly lower compared to the other 3 cultivars. The yields obtained from 'Florida Radiance', Winterstar™ and Sensation™ were not significantly different (Table 2). In 2017, 'Florida Radiance' was the best overall performer in both the OF and the LCHT (Table 2).

Evaluation of strawberry cultivars in the OF (2019–20). The mean yield in the OF ranged from 456 lb/acre (511 kg/ha) for 'Florida Brilliance' to 607 lb/acre (680 kg/ha) for 'Florida Radiance'. Mean yield from 'Florida Radiance' in the OF was higher than the other 3 cultivars but there were no significantly differences among them (Table 3).

In the LCHT, mean yield ranged from 812 lb/acre (910 kg/ha) for 'Florida Beauty' to 1565 lb/acre (1755 kg/ha) for 'Florida Radiance'. The yield obtained from 'Florida Beauty' in the LCHT was significantly lower than the other 3 cultivars. The yields obtained from 'Florida Radiance' was numerically higher than, but not significantly different from 'Florida Brilliance' and Sensation™ (Table 3).

Across the three-year evaluation beginning with the 2016–17 growing season, the best yield performances were observed for

Winterstar™ in the OF and 'Strawberry Festival' in the LCHT. For the 2017–18 growing season, 'Florida Radiance' performed best in both the OF and the LCHT. In 2019–20, 'Florida Radiance' also performed better in the OF and in the LCHT (Table 4 and Table 5, respectively).

Evaluating the OF vs. LCHT. Total strawberry yields by production system was used to help determine the feasibility of growing in a low cost high tunnel vs. open field for small-scale strawberry production in north Florida. Temperatures below 30 °F (–1 °C) can be a common occurrence during the fall and winter months. While strawberry plants can tolerate temperatures near 22 °F (–6 °C) before serious damage to the plant crown occurs, many growers use frost blankets, row covers and latent heat from overhead irrigation to protect the crop from freezes. With the popularity and convenience of growing under cover, many small-scale farmers have begun to grow crops in high tunnels mainly because they provide a layer of protection against frost. A common high tunnel management practice is the closing or rolling down of poly plastic curtains and entry ways. The low cost high tunnel evaluated has pull down sides along both sides of the structure to help insulate the crop against freeze and when pulled-up it acts to ventilate the interior of the structure.

It was observed that during the 2016–17 season there were two major freeze events where temperatures dropped as low as 23 °F (–5 °C). In 2017–18, there were five events reaching as low as 19 °F (–7 °C) and for the 2019–20 season there were three events where temperatures dropped to 26 °F (–3 °C). We believe that the 2017–18 freezing events may have caused plant growth to slow down considerably (Fig. 1). In this case, we conclude that the LCHT did not provide any statistically significant yield advantage over the OF. It must be mentioned however, that the LCHT provided other benefits such as: protection from frost, insect and disease management thereby improving plant health and quality of fruit. However, small-scale and limited resource farmers will still have to balance the additional cost of constructing a LCHT with the benefits it can provide before making a decision regarding whether or not to use it as an alternative production system for organically grown strawberries.

Table 4. Best vs. poor yielding strawberry cultivar performance by production season and production system.

Performance	Season	System	Cultivar[z]
Best	2016–17	LCHT[y]	Strawberry Festival
	2017–18	OF[x]	Fla Radiance
	2019–20	LCHT	Fla Radiance
Poor	2016–17	OF	Fla Radiance
	2017–18	OF	Fla Beauty
	2019–20	OF	Fla Brilliance

[z]For cultivars evaluated by season and production mean values refer appropriately to Table 1, 2, and 3.
[y]LCHT = low cost high tunnel.
[x]OF = open field.

Table 5. Highest yielding strawberry cultivars[z] by production season and production system.

Season	System	Cultivar[z]
2016–17	OF	Winterstar™
	LCHT	Strawberry Festival
2017–18	OF	Fla Radiance
	LCHT	Fla Radiance
2019–20	OF	Fla Radiance
	LCHT	Fla Radiance

[z]For cultivars evaluated by season and production mean values refer appropriately to Table 1, 2, and 3.
[y]LCHT = low cost high tunnel.
[x]OF = open field.

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Comparison of Drought Tolerance in Five Blueberry Cultivars by Using Dehydration Leaf Assay

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Additional index words. dehydration, drought-tolerance

The effect of water stress on the physiological characteristics of two rabbiteye blueberry cultivars ('Premier' and 'Climax') and three Southern highbush cultivars ('Star', 'Rebel', and 'Springhigh') was investigated. A dehydration leaf assay was used to evaluate the drought-tolerance of blueberry types and cultivars. Fully developed leaves were placed in an airtight plastic box containing a salt solution for seven hours. This provided a controlled environment to study leaf water loss. Drought tolerance differed significantly between rabbiteye and southern highbush blueberries cultivars may indicate different sensitivites to dehydration. Despite having the highest stomatal density, 'Rebel' and 'Springhigh' lost the least amount of water, indicating they were least sensitive to dehydration. Application of abscisic acid (ABA) before the experiment decreased the amount of water loss in leaves, indicating the effectiveness of the assay to detect different responses of leaves to dehydration. Water loss was significantly affected by leaf age as younger leaves lost more water compared to intermediate and old leaves. A leaf dehydration assay can be used as a simple and reliable method to compare plant responses to water stress among blueberry cultivars.

Drought stress is one of the major constraints limiting crop production worldwide. The water deficit adversely affects a plant's phenology, development, and reproduction. Drought stress reduces water content, turgor loss, cell growth, leaf water potential, plant growth and development, and yield in the following season (Morales et al., 2013; Hazrati et al., 2016).

Plants respond to drought by stomatal closure to conserve water. Stomata allow for gas exchange by letting in carbon dioxide (CO_2) essential for photosynthesis and regulating water movement through transpiration. Transpiration is affected by water deficiency. In response to a water deficit, guard cells play an important role by controlling transpiration and water loss by stomatal closure. Abscisic acid (ABA) is an important plant hormone which signals stomatal closure under abiotic conditions such as drought.

Southern highbush blueberry (*Vaccinium corymbosum* L.) is the most commercially important and commonly cultivated species of the genus *Vaccinium* in the southern U.S because of low chilling requirements and other adaptive features such as short fruit development periods and increased drought resistance that are inherited from an ancestor species (Lang, 1993). Few studies have focused on the water relations of this species (Lareau, 1989). Highbush blueberry responds very quickly to water stress by reducing transpiration (Mingeau et al., 2001). The rabbiteye blueberry (*V. ashei*) has moderate drought tolerance compared to other crops such as field beans, sorghum, and apples due to a low critical water potential for stomatal closure and low transpiration ratios (Davies and Johnson, 1982). It was also reported that 'Bluegem', a rabbiteye cultivar, had a relatively low critical water potential for stomatal closure and a low transpiration ratio.

There are few studies on the leaf level dehydration response of blueberry plants (Ameglio et al. 2000). Evaluation of drought tolerance in blueberry cultivars in the field requires many years for plant establishment because multiple environmental factors significantly affect plant growth and development. A leaf dehydration assay (Hooper et el., 2014) is a reliable way of phenotyping blueberry cultivars for drought tolerance. The response of leaves in our study was consistent with reported field results. The objective of this study was to compare the relative drought tolerance of five blueberry cultivars based on their response to a leaf dehydration assay.

Materials and Methods

Plant materials. Two to three year old plants of southern highbush cultivars 'Star', 'Rebe', and 'Springhigh', and rabbiteye cultivars 'Climax' and 'Premier', were grown in 3-L pots containing a mixture of coarsely ground pine bark and sand (1:1, vol:vol). Plants were grown in a glass greenhouse with supplemental LED lighting (16 h light) at 28 °C day/18 °C night. A slow-release fertilizer was added in one application after 2 weeks (15 g/pot). Fully developed leaves from each cultivar were selected. The leaves were considered "fully developed" when there was no additional increase in size.

Leaf dehydration assay. Four leaves were detached (with no petiole) from each plant (cultivar) and immediately placed in an airtight plastic box (1.2-L Rubbermaid Takealong© container, Newell Rubbermaid, Atlanta, GA) containing 50 mL of a 333 mM

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NaCl solution. The salt solution was placed in the container and sealed several hours before starting the assay to provide a uniform environment and a constant relative humidity for water loss in each leaf. Then four leaves were placed on a metal wire net on top of the salt solution inside the plastic container. Fifteen seconds later, the leaf was removed from the container and weighed. That measurement took about 5 s per leaf. The leaf was quickly placed back in the box. The leaves were removed from the box every hour to weigh them and then placed back in the box which was re-sealed until the next measurement. The measurement process took 1.5 min for all 20 samples. The measurement was repeated within 7 h. We were careful to prevent spills of the salt solution on the leaves.

Measuring stomatal density. Three fully developed leaves from different plants of each cultivar were used. Clear nail polish was used on three different spots on each leaf. When the nail polish dried, clear tape was used to peel off the nail polish and then it was placed on a microscope slide. A digital camera was used to capture the images under the microscope. ImageJ (National Institutes of Health, Bethesda, MD) (1.47v) was used to count stomata.

ABA application. Commercial ABA (ProTone SL) containing S-abscisic acid at 10% concentration (Valent BioScience Corporation, Libertyville, IL) was applied to both sides of intact fully developed leaves. Leaves were treated with 100 and 500 μM ABA. ABA was dissolved in 1 mL of methanol and then brought up to 1 L with distilled water to make a 1 mM ABA stock solution. Plants were kept in the greenhouse for 4 h after the ABA application. Then leaves were detached and placed in the boxes for the 7-h dehydration assay.

Statistical analysis. Data were analyzed using analysis of variance ($P < 0.05$) using JMP (version 9.0; SAS Institute, Cary, NC) with the fit model procedure. The results are expressed as means ± SE.

Results

A leaf dehydration assay is a simple and fast technique for phenotyping plants. It can be used to compare blueberry response to water deficit stress. We modified the method of Hopper et al. (2014) and changed the measurement interval from 30 min to 1 hour in our study because in our preliminary study we observed very low water loss in blueberry leaf after 30 min compared to grape leaves.

Different responses were observed among blueberry cultivars in our study (Fig. 1). There were significant effects of cultivar ($P \leq 0.0001$) and time ($P \leq 0.0001$) on water loss. There was a significant difference in water loss among two the groups of blueberries (rabbiteye and southern highbush). 'Springhigh', 'Star', and 'Rebel' lost less water per leaf compared to 'Climax' and 'Premier' (Fig. 1). This difference was more obvious especially for 3 h till the end of the 7-h period.

There were highly significant differences ($P \leq 0.05$) in stomata density among the cultivars (Fig. 2). 'Rebel' and 'Springhigh' (rabbiteye) had the highest stomata density compare with the other cultivars ($P \leq 0.05$).

Water loss primarily occurs through the stomata and they respond by closing in the dehydration event. The different observed rates of water loss may be due to different stomatal density in cultivars. However, 'Rebel' and 'Springhigh', which lost the least amount of water, had the highest stomatal density

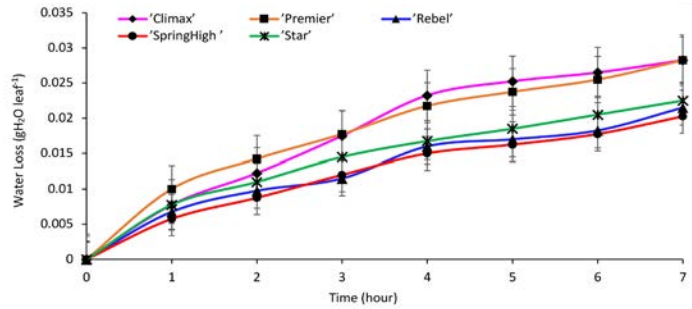

Fig. 1. Water loss per leaf in the dehydration assay. Each data point is presented as the mean ±SE.

while the other three cultivars which had lower stomata density, lost more water. These data showed the exact opposite of what we had prediction if stomatal density were a factor. These results indicated that despite having the lowest stomata density, 'Star', 'Climax', and 'Premier' were slower to respond to dehydration, while 'Rebel' and 'Springhigh' closed their stomata to conserve water more quickly, despite having the largest stomatal density.

Another possible explanation to the differences in water loss among these cultivars may be differences in stomatal sensitivity to ABA, a factor that could be involved in stomatal response to dehydration. To test this hypothesis, different concentrations of ABA (100 and 500 μM) were sprayed on leaves before the dehydration assay. The amount of water lost was significantly ($P \leq 0.0001$) affected by application of ABA (Figs. 3a and 3b). The overall water loss in a 7-h period decreased significantly at 100 and 500 μM ABA compared to the control. These data indicate that leaves responded to an ABA application by closing the stomata. Different responses to the dehydration assay may be attributable to differences in sensitivity of these cultivars to ABA. Different sensitivities to ABA in response to dehydration has also been reported for maize genotypes (Cramer and Quarrie, 2002).

Leaf age may also influence amount of water loss due to difference in stomatal sensitivity. Water loss was significantly ($P \leq 0.05$) affected by leaf age (Fig. 4). Younger leaves lost the greatest amount of water compared to intermediate and old leaves. The results indicated that younger leaves were the most responsive to dehydration.

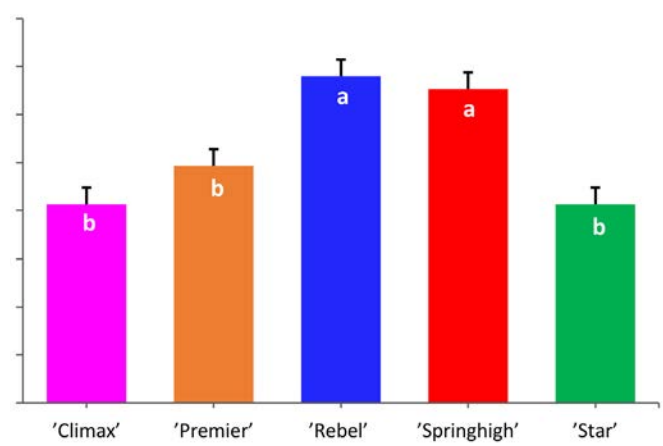

Fig. 2. Stomatal density of five blueberry cultivars. Data are expressed as the mean ± SE.

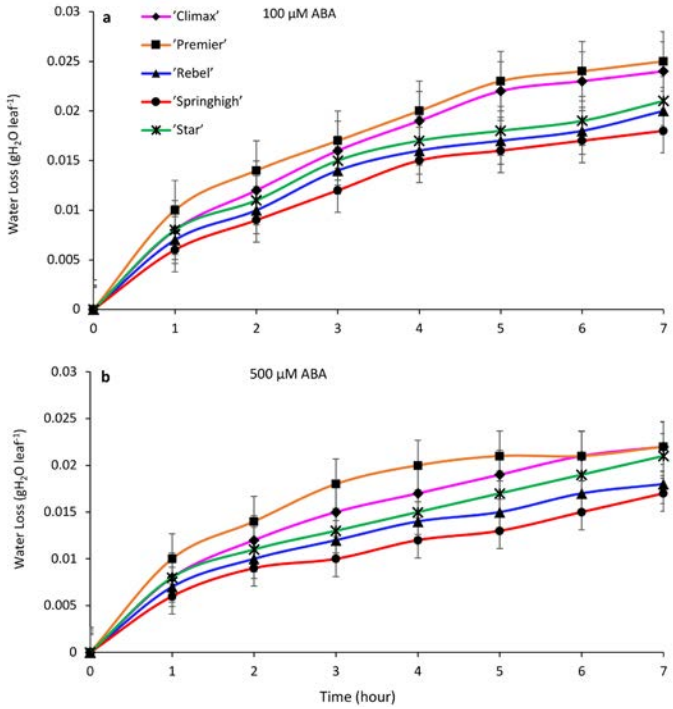

Fig. 3. Water loss per leaf in the dehydration assay after ABA application. Each data point is presented as the mean ±SE. (**a**) 100 μM ABA; (**b**) 500 μM ABA.

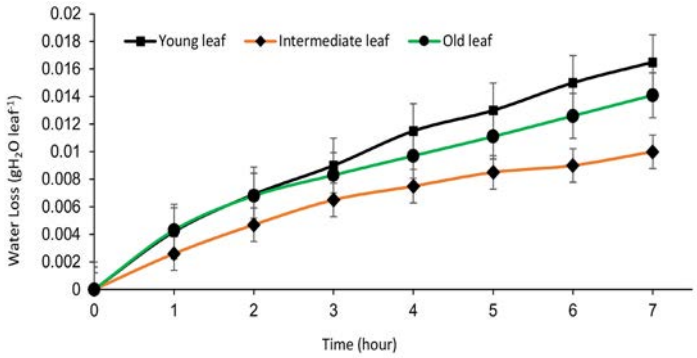

Fig. 4. The effect of leaf age on water loss in the leaf dehydration assay. Young leaves were detached from third to fourth nodes, intermediate from fifth to sixth nodes, and old leaves from tenth to twelfth nodes. Each data point is presented as the mean ±SE.

Interestingly, the data showed that the rabbiteye cultivars ('Premier' and 'Climax') lost significantly ($P \leq 0.0001$) more water compared to southern highbush cultivars ('Star', 'Rebel', and 'Springhigh'), which may indicate that these cultivars are less drought tolerant. This result is consistent with the previous reports of a drought resistance trait in southern highbush blueberries.

Although blueberry can be tested for drought tolerance in field, pot, hydroponic system, and Petri dish studies, most of these methods require that plants to be grown for 4–5 years before testing. Drought tolerance is a complex trait and dependent upon multiple factors. Our results showed that a leaf dehydration assay is a simple, rapid technique to detect differences among blueberry cultivars based on their sensitivity to dehydration.

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—Scientific Note—

Artificial Chilling for Blackberry Production in Florida

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Additional index words. artificial cooling unit; blackberry yield, vernalization

The devastation of Florida's citrus injury caused by citrus greening disease (HLB) has created a need for alternative crops to replace citrus. Blackberry is a crop with some potential to fill this gap, given the experience commercial growers in Florida have with other berry crops. However, blackberry cultivars have variable chilling requirements for productive fruiting (Yazzetti and Clark, 2001). Florida currently has only a small amount of commercial blackberry production, mostly using Arkansas blackberry cultivars, which require more chilling than is available in most of Florida (Andersen and Crocker, 2013). The objectives of this study are to quantify the yield increases possible from additional chilling and to explore the viability of artificial cooling systems to provide needed chilling requirements for Florida blackberry production.

This experiment was a field trial continuation of a trial took place in 2019 at the University of Florida IFAS greenhouses in Gainesville, FL. Two blackberry cultivars commonly grown in Northern Florida, 'Natchez' and 'Ouachita', were used in the trial. Plants from each variety were split into four groups: one untreated control that remained outdoors over the winter, and three treated groups that were artificially chilled in an insulated refrigeration unit at 4 °C for either 250, 500, or 750 h. The treated plants received artificial lighting while in the cooling unit and were placed with the control plants after receiving the prescribed number of chilling hours. After all chilling treatments were applied, the potted plants were grown at a commercial organic blackberry farm near Hawthorne, FL. Fruit were counted and harvested regularly during ripening and yield was recorded.

The yield data from 2020 showed an increase in yield in 'Ouachita' from the control to the 500-hour treatment and a decrease in yield beyond 500 hours. 'Natchez' increased in yield with every increase in chilling hours, although yields of both varieties, and especially 'Ouachita' were lower than they had been in 2019. This is likely the result of significant plant mortality and stunting from cane blight, which affected plants from both varieties, but particularly 'Ouachita'. Despite these setbacks, the data show a similar trend to data from the 2019 trial. In both years 'Ouachita' had the highest yield at 500 chilling hours. In 2019 this was significantly higher than the control but not the 250- or 750-h treatments. 'Natchez' seemed to trend towards higher yields with each additional increment of chilling hours. While this research shows some promising trends, further studies are needed to get a consistent, statistically significant result. Furthermore, the viability of artificial cooling for commercial production is still very much in doubt, and further research should explore its viability and alternatives. Additional research on this topic should be expanded to search for other viable cultivars for Florida production, as well as breeding Florida-adapted cultivars.

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—Scientific Note—

Vanilla Chromosome Counting—Techniques and Implications

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Additional index words. cross-breeding compatability, *Vanilla planifolia*

Vanilla planifolia originated in Central and South America and is now a highly valuable and sought-after spice. Originally transferred internationally by Spanish conquistadors (Childers, 1948), vanilla is now grown worldwide, with major production occurring in Madagascar, Indonesia, Uganda, and India (De La Cruz, et al., 2009). However, the majority of propagation has occurred via cuttings resulting in severely limited genetic diversity of commercial plantings (Sasikumar, 2010). While *V. planifolia* is the primary commercial species, over 100 species of vanilla are classified (Soto and Cribb, 2010). There is significant diversity among vanilla species, not only in physical characteristics, but also in chromosome numbers. We have found differences among and within species. This means that we cannot automatically assume that all vanilla species have the same number of chromosomes, nor can we assume all accessions within a species have the same number of chromosomes. When selecting plants to cross pollinate, we want to ensure that they have the same number of chromosomes to avoid problems with reproductive viability in the future. Inadvertently crossing incompatible plants could waste time and resources on a plant that is vegetatively viable, but is sterile and unable to produce fruit once it reaches maturity. Because of the importance of breeding compatible species, we are developing a reliable method for determining the number of chromosomes in accessions from our diversity collection.

Methods were adapted from previous work by Aliyeva-Schnorr et al. (2015) and Kirov et al. (2014). Material was obtained from the vanilla collection at the Tropical Research Education Center in Homestead, FL. Several accessions of both *V. planifolia* and *V. pompona* species were selected based on desirable traits and physical differences. Apical meristems were harvested from each accession and treated with 1% colchicine for 2 h at RT to arrest cells in metaphase. Cells were washed with water and simultaneously fixed and stained in a 3:1 EtOH:Aceto-Orcein (EtOH:AO) solution for 48 hours at 4 °C. The EtOH:AO solution was removed, cells washed, and the solution replaced with an enzyme solution containing 75 µM KCl solution, 3% Cellulase Onozuka R-10, 1% Pectolyase Y-23, and 3% Hemicellulase. The cells were incubated in the enzyme solution for 75–90 min. at 37 °C. Once digested, the cells were centrifuged and the enzyme solution removed. The cells were washed in 75 µM KCl and the centrifugation step repeated. The KCl solution was removed and replaced with 50 µL of 3:1 EtOH:GAA and the cells were resuspended. An ice-cold slide was placed on moist paper towels on a slide warmer set to 50 °C. The cells were then dropped in 10 µL droplets onto the slide from about 10cm. After the droplet spread and dried, a second droplet of EtOH:GAA was dropped on top of the first droplet and the slide was left on the slide warmer for 2 min on the paper towel followed by 1 min without the paper towel. The slide was removed from the slide warmer, a drop of aceto-orcein stain placed on top of the chromosome spread and a cover slip placed on top of the sample. The cells were viewed at 1000× magnification using an oil immersion lens. (Fig. 1).

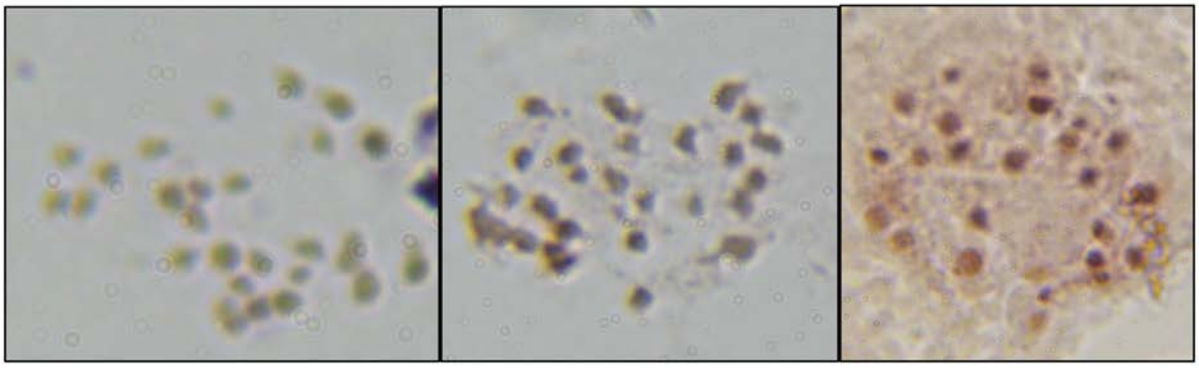

Fig 1. Chromosome spreads from *V. planifolia* specimens at 1000× magnification.

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Using these methods, we were able to identify differences in our collection among different species, but also within species. Among *V. planifolia*, we found the most common number of chromosomes was 28. However, among *V. pompona*, we found either 24 or 28 chromosomes. This technique will allow us to continue screening our collection and determine which species are compatible for cross breeding.

While traditional methods may not always be effective, many methods exist for counting chromosomes. Reliable methods of chromosome counting are crucial to the success of our breeding program and we will continue to learn more about the diversity of our collection as we process more samples.

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—Scientific Note—

Breeding Specialty Vanilla for Production In the United States

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Additional index words. nursery propagation, vanilla cold tolerance, *Vanilla planifolia*

The economic potential of Florida-grown vanilla is immense. *Vanilla planifolia*, the primary commercial species, has been grown in Florida since before 1910. Key limitations to establishing a vanilla industry in Florida have been identified over the last three years since the creation of the vanilla breeding program at the University of Florida Tropical Research and Education Center (UF TREC). The major limitations restricting the vanilla industry today include 1) prohibitively expensive or restricted availability of clean planting material, 2) the lack of optimized horticultural information, and 3) inadequate connections between various stakeholders including government officials, vanilla growers, and vanilla buyers. We are rapidly making progress with each of these challenges as part of Phase 1 for establishing this new industry. In the interim, Phase 2 includes the development of designer vanilla cultivars with known agronomic performance and enhanced genetics through classical breeding. Multiple intraspecific and interspecific vanilla hybrids have been created and verified through seedling selection. Potential traits for improvement include disease resistance, aroma quality, and vigor.

Materials and Methods

A survey was conducted using Qualtrics to identify potential commercial growers and to locate those currently growing vanilla in Florida. This survey included input from extension agents throughout southern Florida. A preliminary screen of *V. planifolia* types identified a particular accession with significantly larger beans than most. This accession was submitted for micropropagation as part of a privately funded project supporting domestic growers (Fig. 1).

Results

There were 195 respondents of the vanilla survey from 64 cities in 25 counties in Florida. 66% of respondents self identified as hobbyists, 25% are interested in commercial vanilla production, and 9% are interested in vanilla nursery propagation. 61, 18, and 23% of respondents indicated they have 1, 1–5, or > 5 acres, respectively. 69% of respondents already grow orchids, 70% grow tropical fruits, and 26% already grow vanilla.

Our vanilla cold tolerance study was postponed until 2021 as a result of Cymbidium mosaic virus being detected in stock

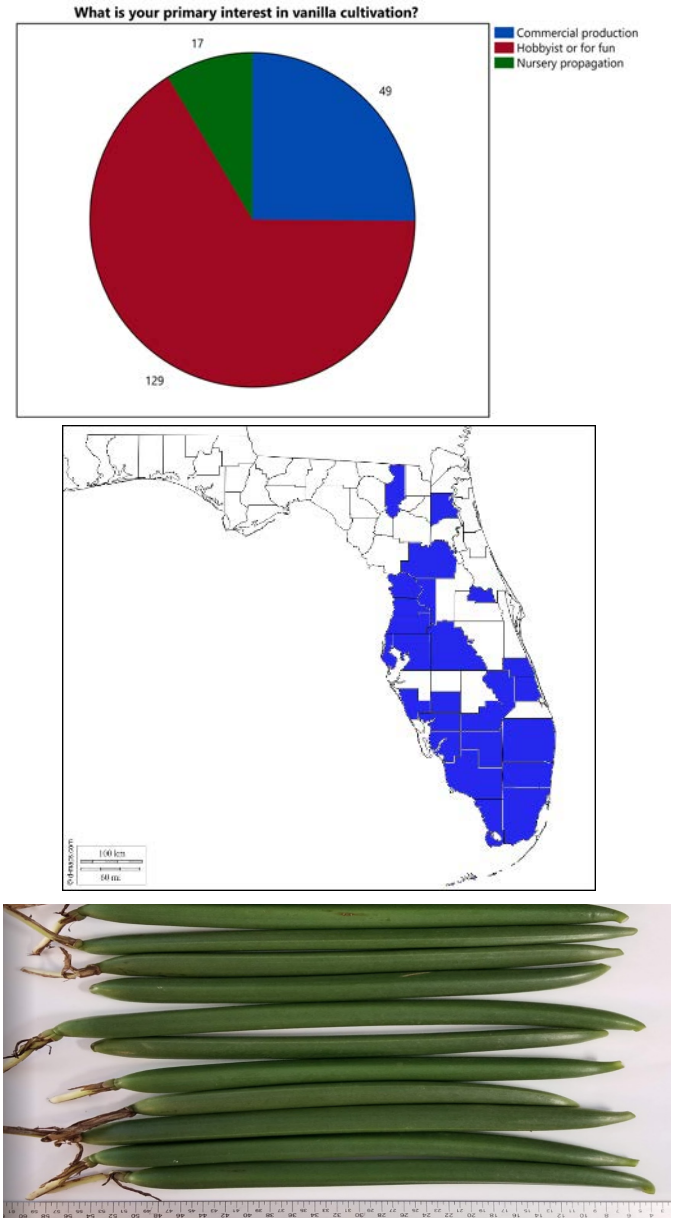

Fig. 1. Survey respondents indicated their interest in vanilla cultivation (top) and location in Florida (center). Florida-grown *V. planifolia* beans are also shown (bottom) from an accession that is part of a micropropagation project.

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plants. Privately funded propagation activities will ensure clean stock material in the future.

Conclusions

Increasing exposure to our vanilla research is elevating the vanilla program. About 400 potential growers in Florida have expressed interest in vanilla cultivation. Surveys have identified that most of these are small acreage growers in southern Florida. The vanilla cold tolerance study scheduled for 2020 has been delayed until 2021 due to nursery plants testing positive for Cymbidium mosaic virus. The ultimate impact of this virus on plant productivity is unknown, but distributing virus-infected plants is not an option. The propagation of an exceptional, Florida-adapted *V. planifolia* will ensure sufficient planting materials for Florida vanilla growers from 2021 into the future.

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—Scientific Note—

Fertigation in Avocado: Monitoring Soil Ions as a Diagnosis in Nutrition

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Mexico is one of the main avocado exporters worldwide. Mexico produces three main varieties—'Hass', 'Criollo', and 'Fuerte'. A few years ago, the main buyer was the United States. However, interest has increased in countries and regions such as Japan, the European Union and Latin America. These new customers are a benefit for producers and for the entire value chain. Most avocado fertilizers are broadcast on the soil surface which can lead to losses of N through immobilization, denitrification and volatilization.

Performance and quality for avocado can be improved if nutrition is optimized, this includes the composition of the nutrient solution, electrical conductivity (EC) and pH. When roots absorb excessive cations, they offset this by excreting protons (H^+), which leads to acidification of the rhizosphere. When they absorb more anions, roots excrete hydroxyl (OH^-), which leads to alkalization of the rhizosphere. Fertigation using the correct combination of water and nutrients can produce high yields and high quality fruits and vegetables.

Fertigation uses both nutrients and water more efficiently than broadcast fertilizer applications and non-targeted water use (such as furrow irrigation). An increase in fertigation frequency can reduce the concentration of immobile macronutrients, such as phosphorus (P), potassium (K), and micronutrients. Nutrients should also not be added during the entirety of long irrigation runs. Micronutrients, may be applied through the irrigation system in chelated form without causing any precipitation. Through good management, it is possible to increase water use efficiency (WUE).

Materials and Methods

The study was carried out on 98.84 acres in the avocado region of Michoacan, Mexico. Extractors of the soil solution "Chupatubos" were installed in the area of the irrigation bulb, at a depth of 60 cm. Evaluations took place during the months of July and August (rains) 2019. Laboratory analysis of soil and water quality was carried out prior to the application of the nutrient solution. The nutrient solution should consider the relationship between the cations ($meq \cdot L^{-1}$) (7 K^+, 9 Ca^{++}, and 4 Mg^{++}) and anions ($meq \cdot L^{-1}$) (12 NO_3^-, 1 $H_2PO_4^-$, 7 $SO_4^=$), the combination that corresponds to electrical conductivity (EC) 2.0 $dS \cdot m^{-1}$.

Results and Discussion

Five samples of the extractors were collected and analyzed with field-specific ion sensors (NO_3^-, K^+, Ca^{++}, Mg^{++}, Na^+, pH, and EC). The concentration of potassium, calcium, magnesium ($meq \cdot L^{-1}$) were below the dose applied by fertigation. There was variation in the dynamics of NO_3^-. Of the five samples analyzed, three were within the target range for the initial nutrient solution preparation. Potassium, calcium, and magnesium were quickly absorbed by the roots of the plants. While the soil nitrate solution is governed by the rapid use of the plant and/or by its rapid infiltration into the soil, both of which are affected by the solubility of the fertilizers. It is necessary to monitor the nutrients in the soil solution applied by fertigation to monitor nutrition programs.

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Exogenous Gibberellic Acid and Cytokinin Effects on Budbreak, Flowering, and Yield of Blackberry Grown under Subtropical Climatic Conditions

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Additional index words. Bramble, dormancy, flower abortion, plant growth regulator, *Rubus*

***Abstract*. In subtropical climates, inadequate winter chill limits blackberry (*Rubus* L. subgenus *Rubus* Watson) production by causing poor and erratic floral budbreak. To compensate for a lack of chilling, bud dormancy-breaking agents must be developed for subtropical blackberry production. Our previous study showed that gibberellic acid (GA_3) promotes budbreak in three blackberry cultivars but has potential negative side effects on floral development in 'Natchez'. 6-benzyladenine (6-BA) is a synthetic cytokinin that can act as an antagonist of gibberellins during floral transition. The objectives of this study were to evaluate cultivar × exogenous GA_3 interactions, characterize dose effects of exogenous GA_3, and examine synergistic effects of GA_3 and 6-BA. Three field experiments were conducted in west central Florida. All spray treatments were applied at the end of the chilling period. In the first experiment, 'Natchez', 'Navaho', and 'Ouachita' were treated with GA_3 at 0 or 99 g·ha^{-1}. Budbreak was promoted by exogenous GA_3 in all three cultivars (0.9% to 4.5% vs. 42.9% to 69.4%), but yield responses varied considerably. Exogenous GA_3 increased the yield of 'Navaho' and 'Ouachita' by 560% to 931%, whereas it induced flower abortion and caused a 15% yield reduction in 'Natchez'. In the second experiment, 'Natchez' was treated with GA_3 at 0, 25, 99, or 198 g·ha^{-1}. Budbreak increased linearly with GA_3, but yield decreased exponentially with GA_3 because of dose-dependent flower abortion. In the third experiment, 'Natchez' was subjected to five treatments: 1) water control; 2) GA_3 spray application; 3) 6-BA spray application; 4) combined spray application of GA_3 and 6-BA; and 5) sequential spray application of 6-BA at 9 days after GA_3 application. Application rates were 99 and 47 g·ha^{-1} for GA_3 and 6-BA, respectively. Exogenous 6-BA suppressed GA_3-induced flower abortion only to a limited extent. As a result, GA_3-containing treatments caused 65% to 83% yield reductions compared with the control (2382 vs. 410–823 g/plant). These results demonstrate that GA_3 is a highly effective bud dormancy-breaking agent for blackberry. However, the drawback of GA_3 is cultivar-dependent flower abortion, which cannot be fully mitigated by 6-BA. The use of GA_3 can be an important management practice for subtropical blackberry production, but its practical implementation must consider cultivar-dependent responses.**

Blackberry (*Rubus* L. subgenus *Rubus* Watson) is a deciduous berry crop grown primarily in temperate regions. In recent years, the global blackberry industry has grown significantly, driven by increased consumer demand, year-round product availability, improved cultivars, and advanced production methods (Clark and Finn, 2014). The leading producers include the United States, Serbia, Hungary, Mexico, and China (Strik et al., 2007). In the United States, blackberry is the fourth most economically important berry crop, generating $697 million in retail sales during 2019 (California Strawberry Commission, 2019). Although blackberry production was traditionally concentrated in the West Coast of the United States, it is currently expanding to the Southeastern United States (Clark and Finn, 2014), where the production acreage increased by 52% (996 vs. 1512 ha) from 2007 to 2017 (USDA, 2017).

Adequate winter chill is a prerequisite for successful commercial production of many temperate fruit crops (Atkinson et al., 2013; Luedeling et al., 2011). For blackberry, buds must be exposed to a certain amount of winter chill to break bud dormancy in spring. This so-called chilling requirement (CR), generally calculated as cumulative hours at temperatures below 7.2 °C, varies from 300 to 900 h among current major floricane-fruiting blackberry cultivars (Carter et al., 2006; Drake and Clark, 2000). In subtropical climates, mild winter temperatures barely satisfy the CR, resulting in incomplete bud development, poor and erratic budbreak, prolonged flowering, nonsynchronous fruit set, and, ultimately, low fruit yield (Fear and Meyer, 1993; Lin and Agehara, 2020). Therefore, commercial blackberry production in Florida is limited mostly to small-scale U-pick operations for the local market (Andersen, 2007). Bud dormancy-breaking agents must be developed to improve the productivity and consistency of subtropical blackberry production.

Bioactive gibberellins (GA), such as GA_3 and GA_4, have important roles in the regulation of dormancy release in many perennial crops (Horvath et al., 2003; Ionescu et al., 2016). The high efficacy of exogenous GA to induce budbreak of fruit and nut crops has been reported for sweet cherry (*Prunus avium* L.) (Cai et al., 2019; Vimont et al., 2018), peach (*Prunus persica* L.) (Chauhan et al., 1961; Donoho and Walker, 1957), pistachio (*Pistacia vera* L.) (Tzoutzoukou et al., 1998), and Japanese apricot (*Prunus mume* Sieb. et Zucc) (Zhuang et al., 2013). For blackberry, however, exogenous GA has been tested by only a few studies. Our previous study found that exogenous GA_3 was highly effective for advancing the onset of budbreak and increasing the yield of 'Ouachita' blackberry grown in central Florida (Lin and Agehara, 2020).

Exogenous GA_3 exerts different effects on floral development depending on the development stage (Yamaguchi et al., 2014; Yamaguchi, 2008). GA application during dormancy can induce budbreak, but application during flowering or fruit setting can result in flower or fruit abortion (Southwick and Glozer, 2000). Our previous study found that exogenous GA_3 applied at the budbreak initiation stage promoted budbreak but caused a nonsignificant yield reduction in 'Natchez' blackberry, suggesting its potential negative side effects on floral development (Lin and Agehara, 2020). Cytokinins act as an antagonist of GA during floral transition in apple (*Malus* ×*domestica* Borkh.) by upregulating the expression of GA degradation genes and repressing the GA signaling pathway (Li et al., 2018, 2019). In lupin (*Lupinus angustifolius* L.), the application of 6-benzyladenine (6-BA), a synthetic cytokinin, at 2 mM completely prevented flower abortion (Atkins and Pigeaire, 1993). Therefore, combined application of GA_3 and cytokinins may provide synergistic effects. In fact, Galindo-Reyes et al. (2004) reported a 421% yield increase in 'Comanche' blackberry treated with both GA_3 and thidiazuron, which is a cytokinin-like substance. However, individual effects of GA_3 and thidiazuron were not evaluated in their study.

The goal of this study was to optimize the application protocol of GA_3 as a bud dormancy-breaking method for subtropical blackberry production. We evaluated cultivar × exogenous GA_3 interactions, characterized dose effects of exogenous GA_3, and examined synergistic effects of GA_3 and 6-BA.

Materials and Methods

Experiment sites and plant material. One field experiment was conducted at the

University of Florida's Gulf Coast Research and Education Center in Balm, FL (lat. 27°76′N, long. 82°23′W; elevation 39 m) during the 2017 to 2018 season (Expt. 1). Three erect, floricane-fruiting cultivars, Natchez, Navaho, and Ouachita, with estimated CRs of 300, 800 to 900, and 400 to 500 h, respectively, were used (Drake and Clark, 2000; McWhirt, 2016). Plants were established in wooden planter boxes (length 3.7 m × width 0.6 m × height 0.3 m) filled with aged pine bark in Apr. 2013. Each plot (experiment unit) consisted of six plants. Plants were spaced at 0.61 m within a row and 1.83 m between rows, and they were grown under a 40% black shadecloth to reduce fruit damage caused by excessive heat and rain. For trailing blackberry canes, we used a three-wire T-trellis system with the upper, middle, and lower wires positioned at 1.5, 1.1, and 0.7 m from the ground, respectively. We pruned floricanes at the ground level after harvesting was completed. Newly emerged primocanes were pruned to five canes per plant and tipped when they reached the top wire. Laterals were trained and tied on the closest wires. Blackberry production and cane management practices recommended in the southeast region were followed (Fernandez and Krewer, 2008).

Two field experiments were conducted at a commercial blackberry farm located in Plant City, FL (lat. 28°03′N, long. 82°19′W; elevation 39 m) during the 2017 to 2018 (Expt. 2) and 2018 to 2019 (Expt. 3) seasons. 'Natchez' blackberry tissue culture seedlings were transplanted in raised beds in the open field in 2015. Each plot consisted of four and five plants for Expt. 2 and Expt. 3, respectively. Plants were spaced at 0.9 m within rows and 3.6 m between rows. Beds were covered with black landscape fabric and north–south-oriented with a planting density of 3237 plants/ha. Plants were trained to a three-wire vertical trellis with the upper, middle, and lower wires positioned at 1.4, 0.9, and 0.4 m from the ground, respectively. Primocanes were tipped to encourage lateral growth when they reached the lowest wire. Primocanes were then tied to the trellis and trained to loop down when they reached the top wire. Floricanes were pruned in early July, immediately after the final harvest.

Treatments and experimental design. All GA_3 and 6-BA treatments in this study were prepared using ProGibb LV Plus with 5.7% active ingredient (a.i.) (Valent Biosciences, Libertyville, IL) and MaxCel with 1.9% a.i. (Valent Biosciences). Immediately before application, treatment solutions were prepared by adding each chemical in water at the concentrations described. All treatments were applied between 9:00 and 10:00 AM using a CO_2-pressured backpack sprayer (model T; Bellspray, Opelousas, LA) equipped with two flat nozzle tips (XR8002; TeeJet Technologies, Wheaton, IL) spaced 0.46 m apart on the spray boom. The spray pressure was 296 kPa and the spray volume was 935 $L \cdot ha^{-1}$.

In Expt. 1, there were six treatments, including three cultivars (Natchez, Navaho, and Ouachita) and two GA_3 application rates (0 and 99 $g \cdot ha^{-1}$ a.i. or 0 and 106 $mg \cdot L^{-1}$ a.i.) in a factorial treatment structure. There were four replicated plots for each treatment that were arranged in a split-plot design with cultivar as the main plot factor and GA_3 application rate as the subplot factor. All treatments were applied on 27 Feb. 2018. The number of accumulated chilling hours at temperatures below 7.2 °C recorded from 1 Nov. 2017 through 27 Feb. 2018 was 199 h.

In Expt. 2, treatments comprised four spray application rates of GA_3 (0, 25, 99, and 198 $g \cdot ha^{-1}$ a.i. or 0, 27, 106, and 212 $mg \cdot L^{-1}$ a.i.). There were five replicated plots for each treatment that were arranged in a randomized complete block design. All treatments were applied on 20 Feb. 2018. The number of accumulated chilling hours at temperatures below 7.2 °C recorded from 1 Nov. 2017 through 20 Feb. 2018 was 227 h.

In Expt. 3, there were five treatments: water control; GA_3 (spray application of GA_3); 6-BA (spray application of 6-BA); GA_3 + 6-BA (combined spray application of GA_3 and 6-BA); and $GA_3 \rightarrow$ 6-BA (sequential spray application of 6-BA at 9 d after GA_3 application). Application rates were 99 and 47 $g \cdot ha^{-1}$ a.i. for GA_3 and 6-BA, respectively. There were four replicated plots for the control and three replicated plots for the other treatments that were arranged in a randomized complete block design. All treatments were applied on 19 Feb. 2019, except for the 6-BA application in the $GA_3 \rightarrow$ 6-BA treatment, which was applied on 28 Feb. 2019. All treatments including the water control were supplemented with a nonionic surfactant (Regulaid; Kalo, Inc., Overland Park, KS) at 0.1% (v/v). The number of accumulated chilling hours at temperatures below 7.2 °C recorded from 1 Nov. 2018 through 19 Feb. 2019 was 201 h.

Floral budbreak and flower number. We selected five representative floricanes per plot before treatments. For each cane, a section containing 20 nodes, starting with the third node from the cane tip, was labeled to monitor budbreak. We counted the number of nodes with budbreak on a mostly weekly basis. Budbreak was defined as the stage at which a green tip was visible. The percentage of budbreak was calculated by dividing the number of nodes with budbreak by 20 and multiplying by 100. Flowers at or above the popcorn stage were counted as flowers (Fernandez, 2013; Hussain et al., 2016).

Fruit yield, size, and quality. Harvesting was performed weekly between 9 May and 5 July 2018 during Expt. 1, between 7 May and 15 June 2018 during Expt. 2, and between 22 Apr. and 14 June 2019 during Expt. 3. Harvested berries were graded based on the U.S. Department of Agriculture (USDA) grade standards (USDA, 2016). Both number and fresh weight of harvested berries were recorded during Expt. 1, whereas only fresh weight was recorded during Expts. 2 and 3.

To determine fruit size and quality, the four largest (by weight) marketable berries were sampled per plot from six peak harvests during Expt. 1 and Expt. 2. For each berry, fresh weight was recorded, and berry length and width were measured across the longest and widest parts, respectively, using a digital caliper. The soluble solids concentration (SSC) was measured using a digital refractometer (PAL-1; ATAGO, Tokyo, Japan) on unfiltered juice. Fruit juice was squeezed from the entire berry with a stainless-steel garlic press.

Statistical analysis. To describe the dose-responses of dependent variables to GA_3 application rates during Expt. 2, we fitted each data set to the following four models by using SigmaPlot (version 14.0; Systat Software Inc., San Jose, CA): linear Eq. [1]; quadratic Eq. [2]; exponential decay Eq. [3]; and exponential plateau Eq. [4]. The best model was selected based on the smallest corrected Akaike information criterion (AICc).

$$y = a + bx \quad [1]$$

$$y = a + bx + cx^2 \quad [2]$$

$$y = a + b\,exp(-kx) \quad [3]$$

$$y = a + b[1 - exp(-kx)] \quad [4]$$

Data were analyzed by the generalized linear mixed model procedure (PROC GLIMMIX) in SAS statistical software (SAS 9.4; SAS Institute Inc., Cary, NC). In Expt. 1, cultivar, GA_3 rate, and cultivar × GA_3 rate interaction were considered fixed effects, and replication and replication × cultivar interaction were considered random effects. In Expt. 2 and Expt. 3, treatments were considered fixed effects and replication was considered a random effect.

Continuous data (yield, berry fresh weight, berry length, and berry width) were modeled using a lognormal distribution (DIST=LOGNORMAL). For the model parameter

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estimation, boundary constraints on covariance were removed (NOBOUND), and degrees of freedom for the fixed effects were adjusted by using the Kenward-Roger degrees of freedom approximation (DDFM=KR). Continuous proportion data (budbreak and SSC) were modeled with the beta distribution (DIST=BETA). If budbreak data were 0%, then they were converted to 1% before the subsequent statistical analysis. Flower count data were modeled with the negative binomial distribution (DIST=NEGBIN) in Expt. 1, and with the Poisson distribution (DIST=POISSON) in Expt. 3. Fruit count data (fruit number) were modeled with the Poisson distribution in Expt. 1. The model selection in these tests was performed based on the smallest AICc.

Budbreak and cumulative flower count data were analyzed using a repeated measures analysis because they were collected repeatedly from the same experimental unit. To identify the appropriate covariance structure, model parameters were estimated by using maximum likelihood estimation based on Laplace approximation (METHOD=LAPLACE) with default bias-corrected sandwich estimators (EMPIRICAL=MBN) (Bowley, 2015). The appropriate covariance structure was selected based on the smallest AICc. When the appropriate covariance structure was chosen, model parameters were estimated using the restricted subject pseudo-likelihood method (METHOD=RSPL), and degrees of freedom for the fixed effects were adjusted using Kenward-Roger degrees of freedom approximation (DDFM=KR2) to control the type I error (Stroup, 2018).

For continuous data, the data were back-transformed by exponentiating the least square means. For continuous proportion data and count data, data were rescaled to the original scale by using the inverse link option (ILINK) in the LSMEANS statement. Least square means comparisons were performed using the Tukey-Kramer test. Unless otherwise noted, P values < 0.05 were considered statistically significant. Back-transformed or rescaled data are reported in this study.

Table 1. Budbreak of 'Natchez', 'Navaho', and 'Ouachita' blackberry grown under subtropical climatic conditions as affected by spray application of gibberellic acid (GA_3) in the 2017–18 season (Expt. 1).[z]

		Budbreak (%)			
Cultivar	GA_3 (g·ha^{-1})	9 Mar. (10 DAT)	15 Mar. (16 DAT)	23 Mar. (24 DAT)	28 Mar. (29 DAT)
Natchez	0	4.3 ab[y]	5.3 bcd	4.2 bc	4.5 bc
	99	46.6 a	54.2 ab	57.8 a	57.7 ab
Navaho	0	0.9 b	0.9 d	0.9 c	0.9 c
	99	14.0 b	31.1 c	42.0 b	42.9 b
Ouachita	0	0.9 b	0.9 d	0.9 c	0.9 c
	99	49.5 a	65.1 a	70.5 a	69.4 a
			P value		
Cultivar		0.0304	0.0743	0.0883	0.1835
GA_3		0.0001	0.0001	0.0001	0.0001
Cultivar × GA_3		0.0001	0.0001	0.0001	0.0001

[z]Plants were treated with GA_3 at 0 or 99 g·ha^{-1} (0 or 106 mg·L^{-1}) via spray application with a spray volume of 935 L·ha^{-1} on 27 Feb. 2018.
[y]Treatment means or pooled data of each main effect (n = 4) in a column with the same letter are not significantly different (Tukey-Kramer test, P < 0.05).
DAT = days after treatment.

Table 2. Flower number, fruit number, and total marketable fruit yields of 'Natchez', 'Navaho', and 'Ouachita' blackberry grown under subtropical climatic conditions as affected by gibberellic acid (GA_3) treatment in the 2017–18 season (Expt. 1).[z]

Cultivar	GA_3 (g·ha^{-1})	Flower no./cane	Fruit number (no./plant)[y]	Marketable fruit yield (g/plant)
Natchez	0	16.9 ab[x]	47.7 bc	197 ab
	99	13.5 ab	38.6 bc	167 ab
Navaho	0	12.4 b	25.5 c	65 bc
	99	42.1 a	151.8 a	429 a
Ouachita	0	5.0 b	9.9 c	26 c
	99	33.9 ab	76.8 ab	268 a
			P value	
Cultivar		0.3515	0.0213	0.0701
GA_3		0.0103	0.0001	0.0001
Cultivar × GA_3		0.0089	0.0001	0.0006

[z]Plants were treated with GA_3 at 0 or 99 g·ha^{-1} (0 or 106 mg·L^{-1}) via spray application with a spray volume of 935 L·ha^{-1} on 27 Feb. 2018.
[y]Harvest was performed nine times between 9 May and 5 July 2018.
[x]Treatment means or pooled data of each main effect (n = 4) in a column with the same letter are not significantly different (Tukey-Kramer test, P < 0.05).

Table 3. Berry size and soluble solids concentration (SSC) of 'Natchez', 'Navaho', and 'Ouachita' blackberry grown under subtropical climatic conditions as affected by spray application of gibberellic acid (GA_3) in the 2017–18 season (Expt. 1).[z]

Cultivar	GA_3[y] (g·ha^{-1})	Berry FW (g)	Berry length (cm)	Berry width (cm)	SSC (°Brix)
Natchez		6.79 a[x]	2.56 a	2.15 a	8.9
Navaho		4.28 b	2.10 b	1.90 b	11.2
Ouachita		4.73 b	2.15 b	1.99 ab	10.7
	0	4.82 b	2.24	1.98	10.0
	99	5.52 a	2.27	2.04	10.4
			P value		
Cultivar		0.0014	0.0007	0.0083	0.1356
GA_3		0.0493	0.6176	0.0879	0.4808
Cultivar × GA_3		0.3546	0.7922	0.1169	0.1192

[z]Because cultivar × GA_3 interaction was nonsignificant, data were pooled by each main effect. Data were collected for four largest marketable berries per plot sampled from six peak harvests. The season average data are presented.
[y]Plants were treated with GA_3 at 0 or 99 g·ha^{-1} (0 or 106 mg·L^{-1}) via spray application with a spray volume of 935 L·ha^{-1} on 27 Feb. 2018.
[x]For each main effect, treatment means (n = 4) in a column with the same letter or no letter are not significantly different (Tukey-Kramer test, P < 0.05).
FW = fresh weight.

Results

GA_3 × cultivar effects on budbreak (Expt. 1). Budbreak was significantly affected by the cultivar × GA_3 interaction (Table 1). The magnitude of GA_3-induced budbreak varied considerably among the tested cultivars. Compared with the respective controls, exogenous GA_3 increased budbreak 10 to 14 times, 16 to 48 times, and 55 to 78 times in 'Natchez', 'Navaho', and 'Ouachita', respectively (Table 1). At 29 days after treatment (DAT), exogenous GA_3 increased budbreak from 4.5% to 57.7% in 'Natchez', from 0.9% to 42.9% in 'Navaho', and from 0.9% to 69.4% in 'Ouachita'.

GA_3 × cultivar effects on fruit number and yield (Expt. 1). Flower number per cane was significantly affected by the cultivar × GA_3 interaction (Table 2). Compared with the respective controls, flower number was increased by exogenous GA_3 by 240% (12.4 vs. 42.1 flowers/cane) in 'Navaho', whereas exogenous GA_3 had no significant effects on 'Natchez' and 'Ouachita'.

Fruit number and marketable fruit yield showed similar responses and were significantly affected by the cultivar × GA_3 interaction (Table 2). Compared with the respective controls, exogenous GA_3 increased marketable fruit yield by 560% (65 vs. 429 g/plant) in 'Navaho' and by 931% (26 vs. 268 g/plant) in 'Ouachita'; however, it caused a nonsignificant 15% yield reduction in 'Natchez' (197 vs. 167 g/plant).

GA_3 × cultivar effects on fruit size and quality (Expt. 1). The data presented in Table 3 and discussed here were pooled by each main effect because they were not significantly affected by the cultivar × GA_3 interaction.

Berry fresh weight was significantly affected by both cultivars and exogenous GA_3 (Table 3). 'Natchez' produced 44% to 59% heavier berries than 'Ouachita' and 'Navaho'. Exogenous GA_3 increased berry fresh weight by 15% (4.82 vs. 5.52 g/berry) compared with the control. Berry length and width showed trends similar to those of berry fresh weight. Fruit SSC was not significantly affected by cultivars and exogenous GA_3.

GA_3 application rate effects on budbreak in 'Natchez' (Expt. 2). Budbreak was nearly zero when plants were treated with GA_3 on 20 Feb. 2018. In the control, the majority of budbreak occurred by 8 DAT: budbreak slowly increased from 24.5% at 8 DAT to 31.1% at 29 DAT (Fig. 1). Budbreak was induced rapidly by exogenous GA_3. At 8 DAT, budbreak showed a linear dose-response, increasing from 24.5% to 58.3% with increasing GA_3 application rates. At 15 to 29 DAT, the dose-response continued to have a similar linear increase, but the slope decreased gradually from 0.15 to 0.09. This change in slope gradient was due to the budbreak that increased gradually in the control and to the dieback of sprouted buds that occurred only in the GA_3 treatments: budbreak at 198 g·ha^{-1} GA_3 decreased from 58.3% at 8 DAT to 50.9% at 29 DAT.

GA_3 application rate effects on fruit number and yield in 'Natchez' (Expt. 2). Flowering laterals without flowers was increased by exogenous GA_3, and this negative side effect was maximized at 198 g·ha^{-1} (Fig. 2A). The dose-response was described by an exponential plateau model: flowering laterals without flowers increased sharply from 1.7% in the control to 55.5% at 99 g·ha^{-1} GA_3 and then increased gradually to 66.4% at 198 g·ha^{-1} GA_3. According to the exponential plateau model, the estimated minimum (the control) and upper asymptote values were 2.5% and 63.6%, respectively.

Flower abortion was induced by exogenous GA_3 in a dose-dependent manner (Fig. 2B). Abortion occurred more severely in nondistal flowers, and some distal flowers remained intact (Fig. 3). The dose-response was described by an exponential decay: flower number per cane decreased sharply in the control from 21.8 to 5.8 at 99 g·ha^{-1} GA_3 and then decreased gradually to 4.2 at 198 g·ha^{-1} GA_3. According to the exponential decay model, the estimated maximum (the control) and lower asymptote values were 21.8 and 4.1, respectively.

Fruit number was decreased by exogenous GA_3 as a result of flower abortion (Figs. 2C and 3C). The dose-response was described by exponential decay: fruit number decreased sharply in the control from 287 to 113 at 99 g·ha^{-1} GA_3 and then decreased gradually to 109 at 198 g·ha^{-1} GA_3. According to the exponential decay model, the estimated maximum (the control) and lower asymptote values were 287 and 110, respectively.

Marketable fruit yield was also decreased by exogenous GA_3 in a dose-dependent manner (Fig. 2D). The dose-response was described by an exponential decay: marketable fruit yield decreased sharply in the control from 2041 g/plant to 477 g/plant at 99 g·ha^{-1} GA_3 and then decreased gradually to 341 g/plant at 198 g·ha^{-1} GA_3. According to the exponential decay model, the estimated maximum (the control) and lower asymptote values were 2037 and 378 g/plant, respectively.

GA_3 application rate effects on fruit size and quality in 'Natchez' (Expt. 2). Berry fresh weight showed a linear dose-response, decreasing from 13.2 to 10.4 g/berry with increasing GA_3 application rates (Fig. 4A). No significant tested regression model was

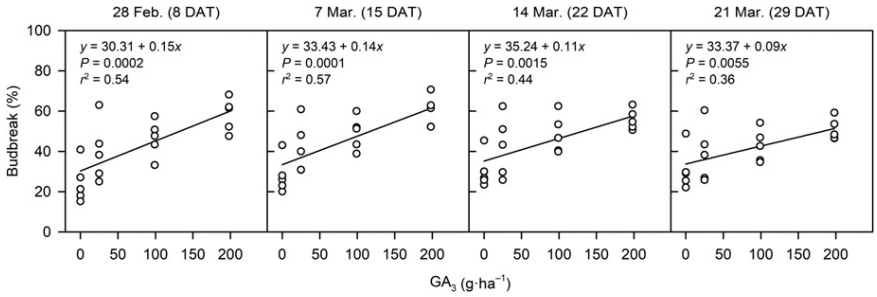

Fig. 1. Linear dose-responses of budbreak to gibberellic acid (GA_3) in 'Natchez' blackberry grown under subtropical climatic conditions in the 2017–18 season (Expt. 2). Plants were treated with GA_3 at 0, 25, 99, or 198 g·ha^{-1} (0, 27, 106, or 212 mg·L^{-1}) via spray application with a spray volume of 935 L·ha^{-1} on 20 Feb. 2018. Budbreak was nearly zero at the time of GA_3 treatment.

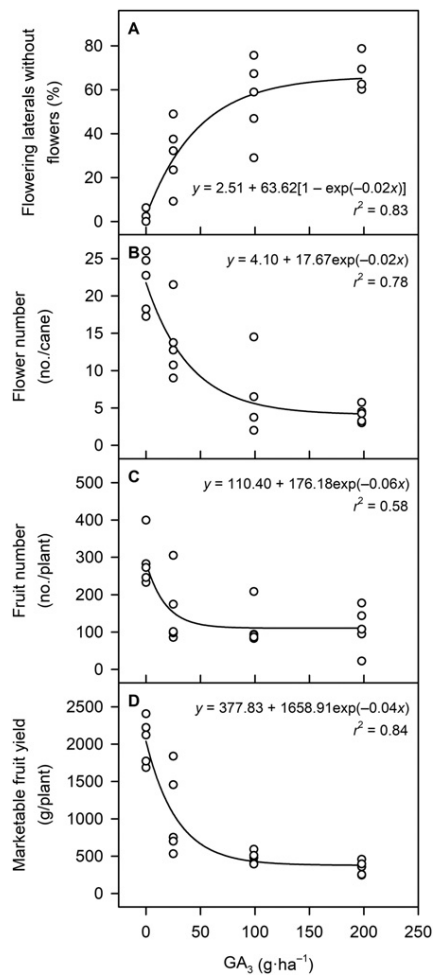

Fig. 2. Dose-responses of flowering, fruit number, and yield to spray application of GA_3 in 'Natchez' blackberry grown under subtropical climatic conditions in the 2017–18 season (Expt. 2). (**A**) Percentage of flowering laterals without flowers. (**B**) Flower number per cane recorded at the end of flowering period. (**C**) Fruit number. (**D**) Marketable fruit yield. Plants were treated with GA_3 at 0, 25, 99, or 198 g·ha^{-1} (0, 27, 106, or 212 mg·L^{-1}) via spray application with a spray volume of 935 L·ha^{-1} on 20 Feb. 2018.

found to describe the relationship between berry length and the GA_3 treatments (Fig. 4B). On average, berry lengths were 3.45. 3.21, 3.40, and 3.36 cm at 0, 25, 99, and 198 g·ha^{-1} GA_3, respectively. Berry width showed a linear dose-response, decreasing from 2.66 to 2.36 cm with increasing GA_3 application rates (Fig. 4C). In contrast, fruit SSC increased linearly from 11.8 to 12.7 °Brix with increasing GA_3 application rates (Fig. 4D).

GA_3 and 6-BA effects on budbreak in 'Natchez' (Expt. 3). At 1 d before treatment (DBT), the budbreak was minimal, ranging from 1.4% to 3.9% in all treatments (Table 4). In the control, budbreak occurred gradually and only partially: budbreak increased from 16.4% at 9 DAT to 37.7% at 24 DAT. All GA_3-containing treatments rapidly induced budbreak to a similar extent. At 9 DAT, the GA_3-containing treatments showed significantly higher percentages of budbreak (83.2% to 86.6%) than the control (16.4%). Similar trends were observed at 16 and 24 DAT. In contrast to the GA_3-containing treatments, the 6-BA treatment had minimal effects on budbreak.

GA_3 and 6-BA effects on flowering and yield in 'Natchez' (Expt. 3). Plants started flowering on 15 Mar. (24 DAT) in all treatments (Table 5). The cumulative flower number per cane initially showed no significant difference among the treatments, ranging from 0.50 to 0.88 and from 2.08 to 4.73 at 24 DAT and 32 DAT, respectively. Thereafter, the control produced flowers steadily: the cumulative flower number increased from 12.20 at 38 DAT to 27.08 at 70 DAT. In contrast, no new flowering occurred in all GA_3-containing treatments. As a result, flower number measured from 52 to 70 DAT was significantly lower in the GA_3-containing treatments than in the control and 6-BA treatment. At the end of the flowering period (70 DAT), the GA_3-containing treatments produced 87% to 92% fewer flowers than the control (27.08 vs. 2.20–3.53 flowers/cane).

Marketable fruit yield was significantly decreased by the GA_3-containing treatments regardless of the 6-BA treatment (Table 5). The plants produced the highest marketable fruit yield in the control, and a comparable yield was obtained in the 6-BA treatment. The GA_3-containing treatments had 65% to 83% lower marketable fruit yield than the control (2382 vs. 410–823 g/plant). Marketable fruit yield also varied between the two treatments containing both GA_3 and 6-BA: the GA_3 + 6-BA treatment had a 101% higher marketable fruit yield than the GA_3 → 6-BA treatment (410 vs. 823 g/plant).

Discussion

Exogenous GA_3 induces budbreak in floricane-fruiting blackberry cultivars. Blackberry is adapted to temperate climates, and

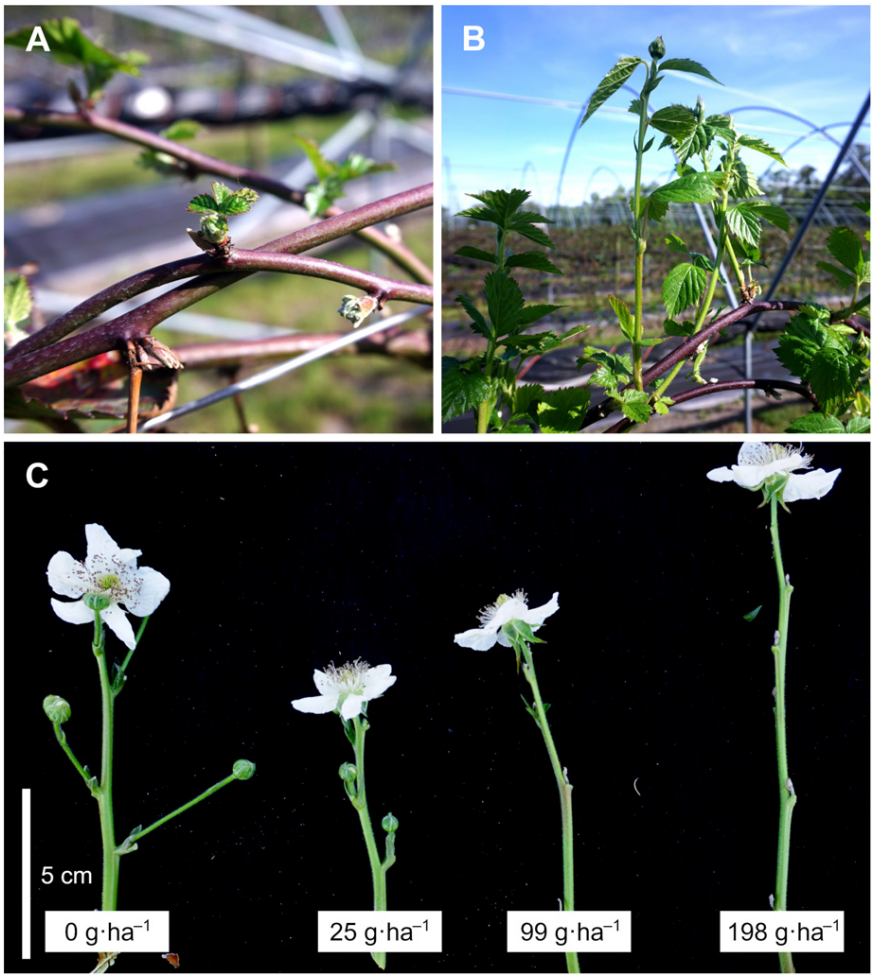

Fig. 3. Budbreak and flowering of 'Natchez' blackberry grown under subtropical climatic conditions as affected by spray application of GA_3 in the 2017–18 season (Expt. 2). (**A**) Budbreak in the control at 15 d after treatment (DAT). (**B**) Budbreak in the GA_3 treatment at 198 g·ha^{-1} at 15 DAT. (**C**) Flowering laterals at 29 DAT. Plants were treated with GA_3 at 0, 25, 99, or 198 g·ha^{-1} (0, 27, 106, or 212 mg·L^{-1}) via spray application with a spray volume of 935 L·ha^{-1} on 20 Feb. 2018.

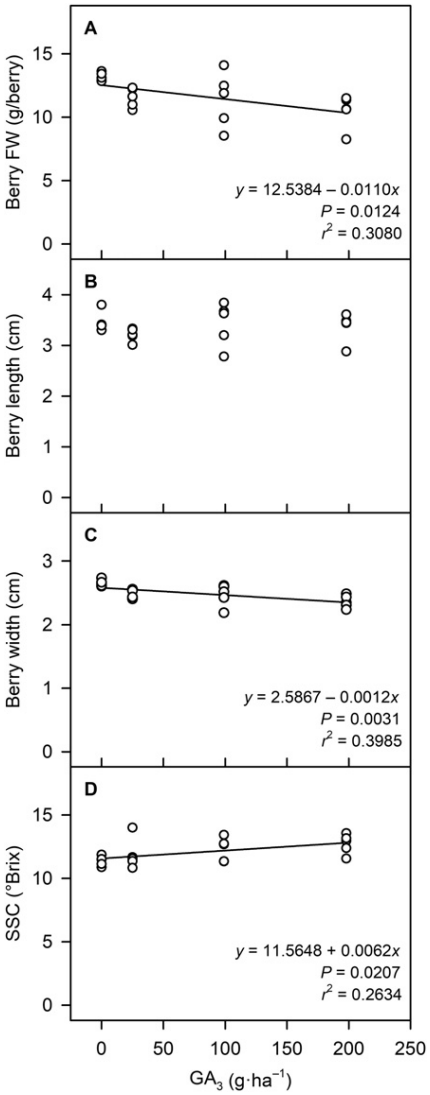

Fig. 4. Dose-responses of berry size and soluble solids concentration (SSC) to spray application of GA_3 in 'Natchez' blackberry grown under subtropical climatic conditions in the 2017–18 season (Expt. 2). (**A**) Berry fresh weight (FW). (**B**) Berry length. (**C**) Berry width. (**D**) SSC. Plants were treated with GA_3 at 0, 25, 99, or 198 g·ha^{-1} (0, 27, 106, or 212 mg·L^{-1}) via spray application with a spray volume of 935 L·ha^{-1} on 20 Feb. 2018. Data were collected for four largest marketable berries per plot sampled from six peak harvests. The season average data are presented.

winter chill has an important role in breaking bud dormancy. Consequently, inadequate winter chill is a major limiting factor for subtropical blackberry production (Lin and Agehara, 2020). GA are phytohormones that act as a signal to break bud dormancy in many perennial crops (Horvath et al., 2003). Major bioactive GA are GA_1, GA_3, GA_4, and GA_7, among which GA_3 is one of the widely used plant growth regulators in horticultural production (Rodrigues et al., 2012; Yamaguchi, 2008). To our knowledge, only one study has examined budbreak induction effects of exogenous GA_3 in blackberry. Galindo-Reyes et al. (2004) reported that the combined spray application of GA_3 at 100 mg·L^{-1} and thidiazuron at 250 mg·L^{-1} increased budbreak from 46% to 81% in 'Comanche' blackberry. However, the tested treatment was a combination of GA_3 and thidiazuron, making it difficult to assess the effects of exogenous GA_3. In this study, a single application of GA_3 at 99 g·ha^{-1} (106 mg·L^{-1}) increased budbreak from 4.5% to 57.7% in 'Natchez', from 0.9% to 42.9% in 'Navaho', and from 0.9% to 69.4% in 'Ouachita' (Table 1), suggesting that GA_3 alone can effectively induce budbreak in floricane-fruiting blackberry. A linear dose-response relationship between GA_3 and budbreak observed in 'Natchez' (Fig. 1) also suggests that budbreak could be further improved by optimizing the application rate.

Budbreak induction by exogenous GA_3 may be associated with antagonistic interactions between GA_3 and abscisic acid (ABA) (Horvath et al., 2003; Ionescu et al., 2016). In many *Rosaceae* fruit crops, GA production is downregulated at the onset of bud dormancy and upregulated during budbreak, whereas the exact opposite trend occurs in ABA production (Ito et al., 2019; Wen et al., 2016). Such dynamics of endogenous GA and ABA levels can be triggered by exogenous GA_3 (Yue et al., 2018; Zhang et al., 2016). In addition, exogenous GA could promote budbreak indirectly by stimulating oxidative stress responses (Beauvieux et al., 2018). Zhuang et al. (2013) found that application of 100 µM GA_4 to Japanese apricot increased budbreak from 20% to 60% and stimulated the production of proteins involved in oxidation-reduction responses.

Cultivar-dependent effects of exogenous GA_3 on flowering and fruit production. In 'Natchez', despite significantly increased budbreak, exogenous GA_3 decreased marketable fruit yield by causing severe flower abortion (Figs. 2B and 3C). Inhibitory effects of GA_3 on floral development are well-recognized in many woody perennial crops (Engin et al., 2014; Facteau et al., 1989; Hoad, 1984; Muñoz-Fambuena et al., 2012). In fact, many studies have reported the high efficacy of GA_3 as a flower or fruit thinning agent for several perennial fruit crops, including apricot (*Prunus armeniaca* L.), Japanese plum (*Prunus salicina* Lindl.), nectarine (*Prunus persica* var. *nucipersica* Schneid.), and peach (García-Pallas et al., 2001; González-Rossia et al., 2006; Southwick et al., 1997; Southwick and Glozer, 2000). It is important to note, however, that GA_3 was applied at the flowering or fruit setting stage in these previous studies. In this study, flower abortion occurred even though GA_3 was applied at the budbreak initiation stage, which was at least 20 d before flowering. This observation suggests that residual GA_3 can remain above the optimum level in the tissue for an extended period, or that exogenous GA_3 can induce the upregulation of GA_3 production at an excessive level.

Interestingly, aborted flowers in the GA_3 treatments were mostly nondistal flowers (Fig. 3). This selective GA_3-induced flower abortion may indicate that absorbed GA_3 is subjected to differential translocation between distal and nondistal flowers, or that the sensitivity to GA_3 differs between distal and nondistal flowers.

It is also important to note that GA_3-induced flower abortion was not observed in other cultivars, suggesting that this phytotoxicity is a cultivar-dependent response. In our previous study, we found that reproductive phenology of floricane-fruiting blackberry varies considerably among the three cultivars. The most remarkable difference is that 'Natchez' has a much shorter interval between budbreak and flowering than 'Navaho' and 'Ouachita' (10 vs. 25–27 d) (Lin and Agehara, 2020). This phenological difference may explain why GA_3-induced flower abortion occurred only in 'Natchez'.

In 'Natchez', GA_3-induced flower abortion at 99 g·ha^{-1} resulted in 15%, 77%, and 74% yield reductions in Expt. 1, 2, and 3, respectively. The variable yield reductions by

Table 4. Budbreak of 'Natchez' blackberry grown under subtropical climatic conditions as affected by spray application of gibberellic acid (GA_3) and 6-benzyladenine (6-BA) in the 2018–19 season (Expt. 3).

	Budbreak (%)			
Treatment[z]	18 Feb. (1 DBT)	28 Feb. (9 DAT)	7 Mar. (16 DAT)	15 Mar. (24 DAT)
Control	2.6	16.4 b[y]	33.7	37.7
GA_3	1.8	86.6 a	88.0	84.2
6-BA	3.9	31.5 ab	40.5	41.3
GA_3 + 6-BA	1.4	83.2 a	84.5	79.3
GA_3 → 6-BA	2.4	85.0 a	80.4	80.7
P value	0.9797	0.0001	0.0010	0.0055

[z]Treatments were as follows: control, GA_3 (spray application of GA_3), 6-BA (spray application of 6-BA), GA_3 + 6-BA (combined spray application of GA_3 and 6-BA), and GA_3 → 6-BA (sequential spray application of 6-BA at 9 d after GA_3 application). Application rates were 99 and 47 g·ha^{-1} for GA_3 and 6-BA, respectively, with a spray volume of 935 L·ha^{-1}. All treatments were applied on 19 Feb. 2019, except for the 6-BA application in the GA_3 → 6-BA treatment, which was applied on 28 Feb. 2019.

[y]Means (n = 3–4) in a column with the same letter or no letter are not significantly different (Tukey-Kramer test, $P < 0.05$).

DBT = days before treatment; DAT = days after treatment.

Table 5. Cumulative flower number and marketable fruit yield of 'Natchez' blackberry grown under subtropical climatic conditions as affected by spray application of gibberellic acid (GA_3) and 6-benzyladenine (6-BA) in the 2018–19 season (Expt. 3).

	Cumulative flower no./cane					
Treatment[z]	15 Mar. (24 DAT)	23 Mar. (32 DAT)	29 Mar. (38 DAT)	12 Apr. (52 DAT)	30 Apr. (70 DAT)	Marketable fruit yield (g/plant)
Control	0.69	4.73	12.20	22.62 a[y]	27.08 a	2382 a
GA_3	0.76	2.20	2.33	2.33 b	2.33 c	615 bc
6-BA	0.50	2.58	5.35	10.20 ab	12.66 ab	2330 a
GA_3 + 6-BA	0.88	2.96	3.53	3.53 b	3.53 bc	823 b
GA_3 → 6-BA	0.57	2.08	2.20	2.20 b	2.20 c	410 c
P value	0.9796	0.4855	0.0015	0.0001	0.0001	0.0001

[z]Treatments were as follows: control, GA_3 (spray application of GA_3), 6-BA (spray application of 6-BA), GA_3 + 6-BA (combined spray application of GA_3 and 6-BA), and GA_3 → 6-BA (sequential spray application of 6-BA at 9 d after GA_3 application). Application rates were 99 and 47 g·ha^{-1} for GA_3 and 6-BA, respectively, with a spray volume of 935 L·ha^{-1}. All treatments were applied on 19 Feb. 2019, except for the 6-BA application in the GA_3 → 6-BA treatment, which was applied on 28 Feb. 2019.

[y]Means (n = 3–4) in a column with the same letter or no letter are not significantly different (Tukey-Kramer test, $P < 0.05$).

DAT = days after treatment.

exogenous GA_3 may be due to the different plant performances among the three experiments. Marketable fruit yield of the control in Expt. 1 was only 8% to 10% compared with Expts. 2 and 3. Therefore, in Expt. 1, the negative effects of GA_3 may have appeared less pronounced because of other yield constraints. Planting density was 8970 plants/ha in Expt. 1, but 3237 plants/ha in Expts. 2 and 3. With the relatively high planting density, plant performance could have been limited because of increased competition for resources, thereby reducing marketable fruit yield on a per-plant basis. Plants were established in 2013 in Expt. 1, but in 2015 in Expts. 2 and 3. In Florida, the productivity of 'Natchez' is reported to decline relatively quickly compared with other cultivars. In fact, the marketable yield of 'Natchez' declined by 47% to 78% from the previous two seasons in Expt. 1, but yield decline was not observed in Expts. 2 and 3 (data not shown).

In contrast to 'Natchez', 'Navaho' and 'Ouachita' had increased marketable yield in response to exogenous GA_3 by 560% and 931%, respectively. Estimated CRs of 'Natchez', 'Navaho', and 'Ouachita' are 300, 800 to 900, and 400 to 500 h (Drake and Clark, 2000; McWhirt, 2016). It appears that the beneficial effects of exogenous GA_3 are more pronounced in high-chill cultivars. Similar results were obtained in our previous study: 'Ouachita' showed the highest yield increase in response to exogenous GA_3 among the same three cultivars (Lin and Agehara, 2020). These results suggest that CRs and potential phytotoxicity are important criteria for determining the suitability of the tested cultivar for exogenous GA_3.

Exogenous 6-BA does not alleviate GA_3-induced flower abortion in 'Natchez'. Antagonistic interactions between cytokinins and GA occur at biosynthesis and during catabolism and have effects throughout the signaling levels during many developmental processes (Weiss and Ori, 2007). It is hypothesized that GA inhibits flowering by antagonizing the inductive effect of cytokinins on flowering in apple (Li et al., 2019; Zhang et al., 2019). During floral bud formation, cytokinin production is upregulated but GA production is downregulated in apple (Li et al., 2019), Japanese pear (*Pyrus serotina* Rehd.) (Banno et al., 1985), mango (*Mangifera indica* L.) (Burondkar et al., 2016), and lychee (*Litchi chinensis* Sonn.) (Chen, 1990). In lupin, exogenous 6-BA at 2 mM during the flowering stage completely prevented flower abortion (Atkins and Pigeaire, 1993). In this study, mitigation of GA_3-induced flower abortion by exogenous 6-BA was observed to a limited extent. Despite having similar percentages of budbreak, the GA_3 + 6-BA treatment produced 101% higher marketable fruit yield than the $GA_3 \rightarrow$ 6-BA treatment (Table 5). The GA_3 + 6-BA treatment also had a 34% higher marketable fruit yield than the GA_3 treatment, although this difference was not statistically significant. In the $GA_3 \rightarrow$ 6-BA treatment, 6-BA was applied 9 d after GA_3, which was likely too late to counteract GA_3-induced flower abortion.

The ratio of cytokinin to GA has an important role in the regulation of bud development. For example, a high cytokinin-to-GA ratio induces floral bud formation, but a low cytokinin-to-GA ratio promotes the development of vegetative buds in apple (Li et al., 2018; Xing et al., 2016). Therefore, the limited efficacy of 6-BA to mitigate GA_3-induced flower abortion in this study may be due to the inadequate application rate of 6-BA relative to GA_3. Further research is needed to test combined applications of 6-BA and GA_3 over a wide range of rates or ratios.

Practical implications for two floricane-fruiting blackberry cultivars: Navaho and Ouachita. Our results demonstrate that spray application of GA_3 at 99 $g \cdot ha^{-1}$ is highly effective for increasing both budbreak and marketable fruit yield under subtropical climatic conditions. Exogenous GA_3 has several key features that enable successful commercial implementation. First, according to the manufacturer, it has a favorable safety profile. Second, its application cost is inexpensive. Based on the price at a local major supplier of agricultural chemicals, one application of the GA_3 product at 99 $g \cdot ha^{-1}$ costs ≈$100 per hectare. Third, it has no negative effects on fruit development and quality. Currently, commercial blackberry production is extremely limited in subtropical climates because of inadequate winter chill. Therefore, GA_3 treatment could be an important management practice for improving the adaptability of current major blackberry cultivars to subtropical climates. With the projected loss of winter chill in temperate fruit production areas (Betts et al., 2011; Luedeling et al., 2009), GA_3 treatment could also become an important adaptation tool for temperate blackberry production to cope with global warming.

It is important to note that precautions should be taken when implementing the use of GA_3. First, GA_3 treatment has cultivar-specific phytotoxicity. Because of severe flower abortion, GA_3 treatment is not recommended for 'Natchez'. Before testing GA_3 treatment on new cultivars, the optimum application rate should be determined based on budbreak induction and potential phytotoxicity. Second, to avoid freeze damage to developing flowers, GA_3 should be applied when the maximum chill accumulation is achieved and the risk of freeze damage is minimal.

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Influence of Elevated Manganese Rates on Growth Parameters, Nutrient, and Biomass Accumulation of HLB-affected Trees In Florida

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Enhancing nutrient uptake and tree health play an important role in managing huanglongbing (HLB) affected citrus trees in Florida. A greenhouse experiment was established to evaluate the effect of varying rates of manganese (Mn) on growth and development of 1-year-old HLB-affected sweet orange (*Citrus sinensis*) trees in October 2018 at the University of Florida IFAS (UF/IFAS)Citrus Research and Education Center in Lake Alfred, FL. Fifty percent of the trees were graft-inoculated with the HLB pathogen and the rest of the trees were used as the HLB-free (NHLB), control trees. Four treatments were applied on both sets of the trees in a randomized complete block design with 7 replicates. Data including trunk diameter, tree height and leaf samples were collected, processed and analyzed from trees treated with from four treatments, 0.0 kg·ha^{-1} Mn (Control), 5.6 kg·ha^{-1} Mn (1×, the UF/IFAS recommended rate), 11.2 kg·ha^{-1} Mn (2× UF/IFAS recommended rate), and 22.4 kg·ha^{-1} Mn (4× UF/IFAS recommended rate) on HLB- and NHLB-affected citrus trees. All the other essential nutrients were maintained at current recommendations in all the treatments. Leaf Mn concentrations, tree height and trunk diameter were analyzed by year with repeated measures in SAS-GLIMMIX. Leaf-Mn concentrations were significantly different ($P < 0.0001$) among Mn rates, and across sampling times in both HLB and NHLB trees in 2019. In both HLB and NHLB-affected trees, the Mn rate of 22.4 kg·ha^{-1} Mn recorded the highest leaf Mn concentration with the least square mean (LSM) of 1131 μg·g^{-1} of dry weight. Tree height and trunk diameter presented significant differences across sampling times in both HLB- and NHLB-affected trees in 2019. The 11.2 kg·ha^{-1} Mn rate increased tree height in HLB-affected trees across sampling-time, while the 22.4 kg·ha^{-1} Mn rate increased the trunk diameter of HLB-affected trees. The results presented in this study show that HLB-affected trees would require higher Mn concentrations than healthy trees (NHLB), for similar physiological functions. The results from this study support higher Mn treatments; specifically, 11.2- and 22.4-kg·ha^{-1} Mn (2× and 4×, respectively) than the UF/IFAS recommendation for better tree growth in HLB-affected trees.

During the past decade, the total citrus production in the United States has declined significantly (Blauer, 2020). Florida, the second-largest citrus producer in the country, has recorded the greatest reduction of more than 70%, from 13.5 million tons in 1998 to about 3.5 million tons in 2019 (Blauer, 2020; USDA, 2019). Despite this reduction, citrus remains the leading tree crop produced in Florida, contributing about $9 billion annually to the state's economy, and employing about 45,000 workers (USDA, 2019). Citrus production decline has been attributed to many causes, primarily huanglongbing (HLB), which was first reported in Florida around 2005. The disease was first found in China in the 19th century and has now spread to most parts of the world thus threatening the citrus industry (Blauer, 2020; Hall and Gottwald, 2011; USDA, 2019).

The HLB is caused by a phloem-limited bacterium called *Candidatus* Liberibacter asiaticus (*CLas*) and spread by an insect vector *Diaphorina citri* (Asian citrus psyllid, ACP) (Gilani et al., 2019; Gottwald et al., 2012; Hijaz et al., 2016; Killiny and Nehela, 2017). The insect-vector pierces through the phloem where it introduces the *CLas*. Once the is in the phloem, the plant blocks the phloem as a defense response and translocation of nutrients occurs (Spreen et al., 2014). One of the effects of the latter is a decline in roots and fibrous root density, which leads to a reduction in nutrient and water uptake, hence, a decrease in citrus yield (Graham et al., 2013; Hamido et al., 2017; Kadyampakeni et al., 2014b, 2014c; Killiny and Nehela, 2017). Since the disease has no cure, the management programs adapted are: intensive chemical control of the ACP, aggressive removal of HLB-affected trees, severe pruning, planting disease-free nursery stock (Hall and Gottwald, 2011; Rouse, 2017) and balanced-nutrition (Vashisth and Kadyampakeni, 2020). But the latter has called for much attention because balanced-nutrition tend to improve tree canopy and yield (Morgan et al., 2016; Nwugo et al., 2013; Rouse et al., 2017; Spann and Schumann, 2009).

Currently, there are no guidelines to determine optimal manganese (Mn) concentration in HLB-affected citrus trees in Florida. These guidelines would enable us to better understand the role Mn plays in the nutrition of HLB-affected trees. The University of Florida's Institute of Food and Agricultural Sciences (UF/IFAS) has updated its citrus nutrition guidelines (Morgan and Kadyampakeni, 2020) to guide farm managers and research

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scientists on balanced citrus nutrition with essential nutrients and their relationship to productivity. However, there is insufficient information on how much more Mn the HLB-affected trees require to maintain balanced levels in tissues of the tree. This study sought to generate information on how much Mn the HLB-affected trees require.

Manganese is an essential plant nutrient present in most plant metabolic processes, particularly in photosynthesis (Millaleo et al., 2010). The form of Mn taken up by plants is Mn^{2+}, a reduced form of Mn. Studies done by most researchers show that Mn is taken up by an active transport system in epidermal root cells, where it is transported by the xylem as Mn^{2+} into the plant system (Gherardi and Rengel, 2004; Marschner, 1995; Millaleo et al., 2010; Pittman, 2005). According to Graham (1979), Mn plays three major roles in the plant system: it assists the movement of electrons in photosynthesis, it affects the reduction of nitrate in nitrogen metabolism, and it acts as a precursor for aromatic amino acids and hormones as in auxins, phenols and lignin (Clarkson, 1988). Insufficient Mn in the plant triggers deficiency symptoms, which are usually observed when plant growth is extremely depressed. However, the productivity of plants is already affected when deficiency symptoms are observed (Schmidt et al., 2016). Zekri and Obreza (2012) reported that Mn deficiency in citrus might significantly reduce yield and fruit quality (Zekri and Obreza, 2012). Deficiency symptoms are observed in newly emerged leaves because Mn is immobile in the phloem, and as a result the remobilization of Mn from older to younger leaves is not possible (Schmidt et al., 2016).

Marschner (1995) mentioned that high levels of Mn could be toxic to biological cells, and intensity of the toxicity will depend on the excess Mn concentration in the plant (Lambers et al., 2015; Marschner, 1995). Finding the level of Mn at which HLB-affected citrus trees may be tolerant is deemed necessary, most importantly, for competition between Mn and other essential nutrients. This is because antagonism between Mn and other mineral elements has been reported; where excess Mn seems to limit the uptake of iron, molybdenum and magnesium, which are equally important for plants growth and development (Rietra et al., 2017).

Even though the mechanism at which HLB-affected trees metabolize Mn is not well understood, elevated levels of Mn has proven to correct deficiency symptoms and reduced *CLas* bacterial titers (Zambon et al., 2019). However, it is still not very clear how much the HLB-affected trees require Mn to maintain optimal concentration. Therefore, the objective of this study was to evaluate the effect of varying rates of manganese (Mn) on the growth and development of 1-year-old HLB-affected *Citrus sinensis* 'Valencia' trees under greenhouse conditions in Florida.

Materials and Methods

Site description and trial establishment. This study was conducted for two years in the greenhouse at the UF/IFAS Citrus Research and Education Center (CREC) in Lake Alfred, FL (Latitude 28°5'37"; Longitude 81°43'30"), to evaluate varying Mn rates on one-year old 'Valencia' (*Citrus sinensis*) trees on *Kuharske citrange* rootstock (*Citrus sinensis* × *Poncirus trifoliata*). The trees were planted in 8.7-L nursery containers. Fifty percent of the trees were graft-inoculated with the HLB-causal pathogen *CLas*. At least 3 leaves were grafted to enhance the probability of *CLas* inoculation. Trees were then left for 3 months for infection establishment. Composite soil samples were collected and analyzed for soil pH and Mehlich-3 extractable nutrients to correct for any deficiency.

Initial measurements of tree height and trunk diameter were taken from each plant before treatments were applied. At the same time, leaves were sampled and analyzed for Leaf-Mn and other essential nutrients. Treatments were then applied once confirmed for *CLas* inoculation using a quantitative polymerase chain reaction analysis (qPCR—Applied Biosystems 7500 Fast Real-Time PCR System; Thermo Fisher Scientific). Water was supplied to each container using a drip irrigation system with pressure-compensating drip emitters at a rate of 2 $L \cdot h^{-1}$ (MaxiJet, Dundee, FL) and trees were provided with all essential nutrients according to the UF/IFAS nutritional guide for citrus production (Morgan and Kadyampakeni, 2020).

Treatment application. Four treatments were applied in total; four treatments including the control for each of the HLB and NHLB-affected trees. Each treatment had a seven single tree replication, arranged in a randomized complete block design (using HLB and NHLB as blocks). The treatments comprised of soil application of varying rates of Mn from manganese (II) sulfate monohydrate ($MnS0_4 \cdot H_2O$, 16% Mn). All treatments were applied and mixed within 5-cm depth of the soil by hand, three times a year. Treatments were as follows:

1. Control: 0.00 $kg \cdot ha^{-1}$ Mn; trees that received this treatment were only supplied with other essential plant nutrients apart from Mn.
2. Standard UF/IFAS recommendation (hereafter denoted 1×): 5.6 Mn; this treatment represents the standard practice for Mn fertilizer application on citrus in Florida.
3. Two times the standard rate (hereafter denoted 2×): 11.2 $kg \cdot ha^{-1}$ Mn; this treatment represents a twice the standard recommendation rate.
4. Four times the standard rate (hereafter denoted 4x): 22.4 $kg \cdot ha^{-1}$ Mn ; this treatment represents four times the standard recommendation rate.

Tree height and trunk diameter. Initial tree height and trunk diameter were recorded for each replicate before treatments were applied. Subsequently, in every three-month interval, a measuring pole height stick (model 807396 by SOKKIA Corp., Olathe, KS) and a digital caliper were used to measure tree height and diameter for each replicate in each treatment. The digital caliper recorded the trunk diameter in the North-South (NS) and East–West (EW) directions of the tree. The results were then averaged and converted into cross-sectional area assuming a circular shape. In terms of tree height and trunk diameter, relative growth was estimated for each replicate by subtracting the first measurements (before treatments were applied) from the subsequent measurements (after treatments were applied) for each measurement period.

Leaf and soil sampling. Leaf samples were collected for each replicate before treatments were applied and afterwards every three months, following treatment application. About 15 fully expanded leaves aged 4–6 months were sampled. Immature-, abnormal-appearing-, and dead-leaves were avoided during every sampling period. The leaves were hand-washed immediately after sampling to remove any surface contamination. Sampled leaves were put into clean paper bags and dried in a ventilated oven at 65 °C for at least 72 h (Morgan and Kadyampakeni, 2020). After drying, leaves were ground with Thomas Type Lab Willey Grinder (PSAW-180, Swedesboro, NJ) with a 20-mesh sieve. Leaf tissue samples were then sent to Waters Agricultural Laboratories, Inc.

Table 1. Effects of manganese (Mn) rates on leaf-Mn concentration, tree height, trunk diameter, and soil Mn for huanglongbing (HLB) and healthy (NHLB) 1-year-old 'Valencia' (*Citrus sinensis*) trees after 12 months of treatment application in 2019.

		Leaf Mn concentration		Tree height		Trunk diameter		Soil Mn	
Tree	Mn Rate	Mean ± SE[x]	DF	Mean ± SE	DF	Mean ± SE	DF	Mean ± SE	DF
	--(kg·ha^{-1} Mn)--	--(µg·g^{-1} dry wt)--		--(cm)--		--(cm)--		--µg·g^{-1} dry wt--	
[z]HLB	0	26 d[w]	48	56 ± 6.96 bc	50.92	0.57 ± 0.053 bc	48	154 ± 442 c	48
	5.6	142 c	48	50 ± 6.96 bc	50.92	0.61 ± 0.053 bc	48	1674 ± 442 bc	48
	11.2	502 b	48	68 ± 6.96 ab	50.92	0.58 ± 0.053 bc	48	2976 ± 442 b	48
	22.4	1131a	48	50 ± 6.96 bc	50.92	0.66 ± 0.053 ab	48	5194 ± 442 a	48
[y]NHLB	0	38 d	48	48 ± 6.96 bc	50.92	0.63 ± 0.053 ab	48	700 ± 442 c	48
	5.6	185 c	48	66 ± 6.96 ab	50.92	0.61 ± 0.053 bc	48	3288 ± 442 ba	48
	11.2	713 b	48	57 ± 6.96 ab	50.92	0.58 ± 0.053 bc	48	2017 ± 442 bc	48
	22.4	1978 a	48	55 ± 6.96 ab	50.92	0.63 ± 0.053 ab	48	3570 ± 442 ba	48

[z]HLB = Huanglongbing affected trees.

[y]NHLB = healthy trees.

[x]SE = standard error of the mean, DF = denominator degrees of freedom.

[w]Different letters indicate statistically significant differences among the studied treatments and same letters indicate no significant differences between them. Statistical significance is set at $P < 0.05$.

(Camilla, GA) to determine elemental concentrations of selected nutrients, including Mn, using the acid digestion method. Soil samples were collected at a depth of 15 cm at the beginning of the study and at the end of the 2-year study period. The samples were then dried in a ventilated oven at 100 °C for at least 24 h. The soil samples were then sent to Waters Agricultural Laboratories, Inc. (Camilla, GA) where they were analyzed using inductively coupled plasma atomic emission spectroscopy (ICP-AES), for Mehlich III extractable Mn and other nutrients.

Data analysis. Linear mixed model methodology as implemented in SAS PROC GLIMMIX, SAS 9.4 (SAS Institute Inc, 2018), was used to analyzed response data and determine optimal thresholds. The statistical significance level was established as $P < 0.05$ in the experimental design for all measured parameters. Leaf Mn concentrations, relative tree height, and trunk diameter were analyzed by year with repeated measures. An unstructured variance structure was fitted to the repeated measures. A Kenward-Roger type adjusted test was used to account for any bias in cases where the linear mixed model had missing covariates. The mixed model included fixed effects for treatment, blocks, time, and their interactions. A least significant difference (LSD) means separation was used to determine treatment differences for all response data. Leaf surface area, soil Mn concentration, and above-and below-ground biomass were also analyzed using linear mixed models in SAS. An optimal Mn rate was determined on all statistically significant parameters by mixed model polynomial regression in SAS.

Results and Discussion

Effect of Mn treatment on leaf Mn concentration. Leaf Mn concentrations were significantly different among Mn rates and across sampling times in HLB-affected and NHLB trees in 2019 (Fig. 1). The leaf Mn concentrations recorded for both HLB- and NHLB-affected trees that received 2× and 4× the standard rate were above the optimal Mn concentrations for citrus trees (Zekri and Obreza, 2012). It is also important to mention that these values are true only for NHLB-affected trees, as the optimal Mn range values for HLB-affected citrus trees are unknown. Concentration levels for the 2× UF/IFAS rate in HLB-affected trees presented a concave-up shape, suggesting a tendency for further increase with time (Fig. 1). At a similar Mn rate of 11.2 kg·ha^{-1} (2× UF/IFAS), leaf Mn concentrations in the HLB-affected trees tended to be higher relative to concentrations in NHLB-affected trees (Fig. 1). This result agrees with the Zambon et al. (2019) study, where they found that elevated Mn rates restore some biological functions due to increased metabolism experienced by HLB-affected trees. Although the two higher Mn rates (2× and 4×)

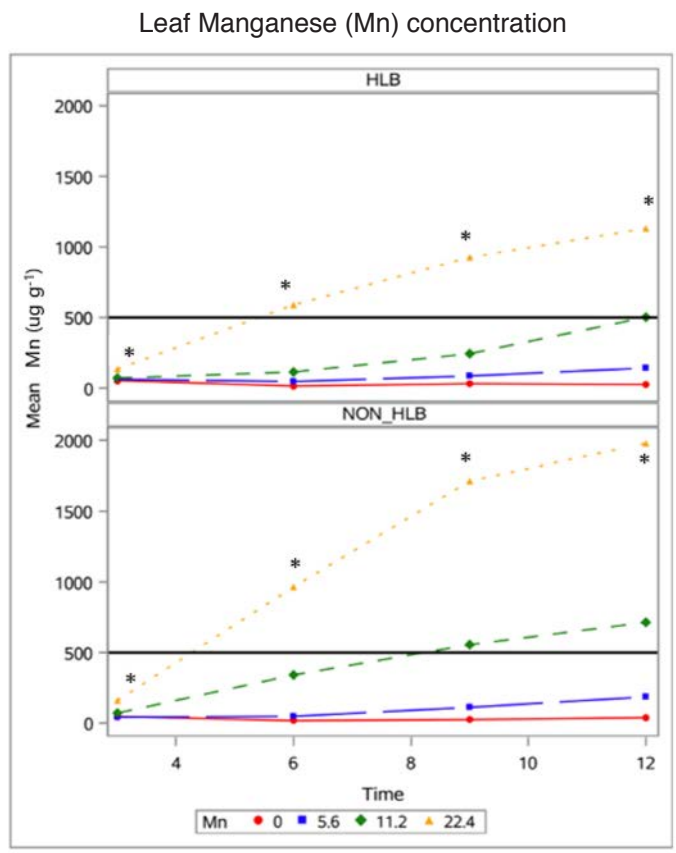

Fig. 1. Leaf manganese (Mn) concentrations across different sampling times (3-, 6-, 9-, and 12-months) after treatments application in 2019, showing the impact of 0.0 kg·ha^{-1} (Control), 5.6 kg·ha^{-1} (University of Florida Institute for Food and Agricultural Sciences recommendation), 11.2 kg·ha^{-1} (2× UF/IFAS rate), and 22.4 kg·ha^{-1} (4× UF/IFAS rate) on HLB-affected and non HLB-affected 1-year-old 'Valencia' (*Citrus sinensis*) trees. Data presented are the least square means and asterisks (*) represents significant differences among treatments ($P < 0.05$).

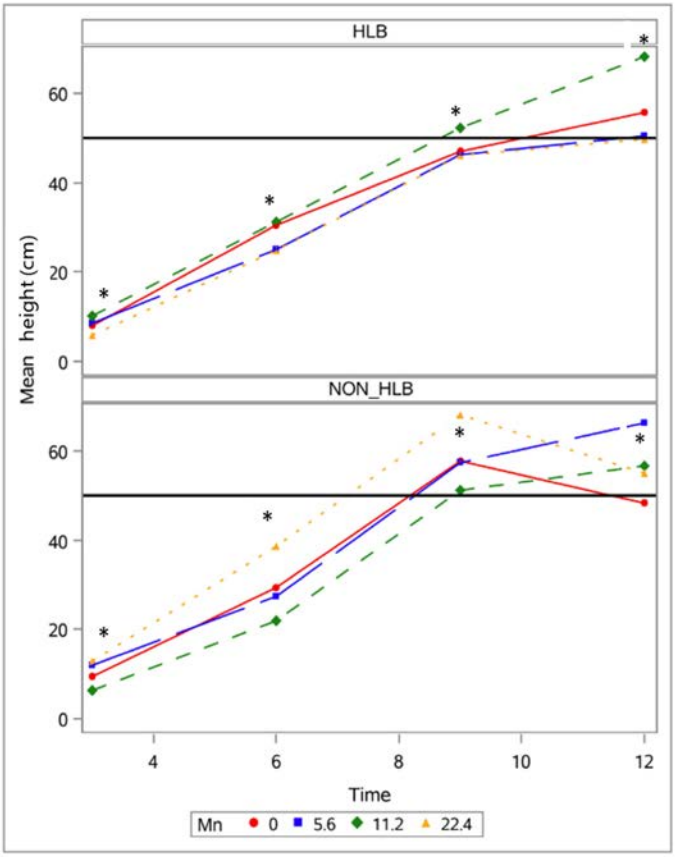

Fig. 2. Effect of Mn treatments 0.0 kg ha^{-1} (Control), 5.6 kg·ha^{-1} (University of Florida Institute for Food and Agricultural Sciences recommendation), 11.2 kg·ha^{-1} and 22.4 kg·ha^{-1} on height of HLB- and non-HLB-affected 1-year-old 'Valencia' (*Citrus sinensis*) trees across different sampling times (3-, 6-, 9-, and 12-months), after treatments application in 2019. Data presented are the least square means and asterisks (*) represents significant differences between sampling time ($P < 0.05$).

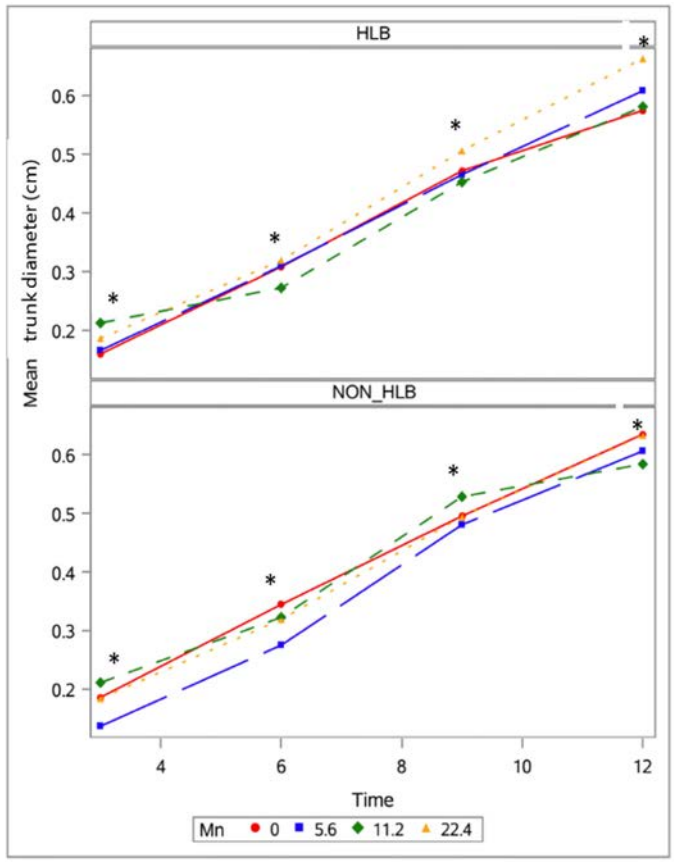

Fig. 3. Effect of Mn treatments 0.0 kg·ha^{-1} (Control), 5.6 kg·ha^{-1} (University of Florida Institute for Food and Agricultural Sciences recommendation), 11.2 kg·ha^{-1} and 22.4 kg·ha^{-1} on trunk diameter of HLB- and non-HLB-affected 1-year-old 'Valencia' (*Citrus sinensis*) trees across different sampling times (3-, 6-, 9-, and 12-months), after treatments application in 2019. Data presented are the least square means and asterisks (*) represents significant differences between sampling time ($P < 0.05$).

used in this study accumulated excess leaf Mn concentrations compared to the standard UF/IFAS recommended rate, there was no visual evidence of toxicity observed in HLB-affected trees.

It is well known that HLB-affected trees have limitations in water and nutrient absorption due to over 70% root loss (Graham et al., 2013; Kadyampakeni et al., 2014a). For this reason, HLB-affected trees may have minimal capacity of acquiring the required Mn through their root system, as is the case of NHLB-affected trees when given the current UF/IFAS recommended rate of 5.6 kg·ha^{-1} Mn (1×). Therefore, the results from this study agree with Zambon et al. (2019), that Mn rate must be increased for HLB-affected citrus trees. An update of Mn rate for HLB-affected trees may be necessary considering that Mn deficiency is detrimental to photosynthesis, and sometimes occurs without visual leaf symptoms (Marschner, 1995; Schmidt et al., 2016).

Effect of Mn treatment on tree growth and trunk diameter. Tree height and trunk diameter were not significantly different among Mn rates (Figs. 2 and 3) for both HLB- and NHLB-affected trees in 2019. However, tree height and trunk diameter showed significant differences across sampling time. The results support the claim that HLB-affected trees show more tolerance to excess Mn when compared to NHLB-affected trees (Fig. 2). For example, as tree height for treatments in NHLB-affected trees peaked in the 9th month, tree height for HLB-affected trees recorded their highest values in the 12th month (Fig. 2). In the Zambon et al. (2019) study, they reported that HLB-affected trees are tolerant to excess Mn levels, which happens to be harmful to *CLas*. Although our study does not present data to support the direct impact of higher Mn levels on *CLas*, our results agree that the HLB-affected trees are tolerant to rates of 2× and 4× the standard UF/IFAS recommended Mn rates (Figs. 2 and 3), as trees show no visual signs toxicity.

The trend of tree height for all treatments in NHLB-affected trees showed quadratic model. However, the trend in HLB-affected trees better fit a linear model suggesting increasing capacity for Mn applications (Fig. 2). This suggests that HLB-affected trees may require more Mn, as they still tend to grow more. Across all sampling-time in HLB-affected trees, tree height was highest at Mn rate of 11.2 kg·ha^{-1} Mn , which is 2× the UF/IFAS recommended rate (Fig. 2). There are mixed results when it comes to research on enhanced nutrition for HLB-affected trees because some researchers found no difference between trials with enhanced Mn treatments and the control plots (Gottwald et al., 2012), others reported a positive impact of balanced Mn and other micro-nutrient treatments (Morgan et al., 2016; Rouse et al., 2017; Zambon et al., 2019). The results from this study

support an increase in tree height and trunk diameter with time for HLB-affected trees, when treated with elevated levels of Mn.

Conclusion

The results of this study support higher than the standard Mn treatments (2× and 4×, 11.2- and 22.4-kg·ha^{-1}, respectively) can be beneficial for HLB-affected trees. The rate of 11.2 kg·ha^{-1} Mn increased tree height in HLB-affected trees across sampling time, while the 4× rate of 22.4-kg·ha^{-1} increased the trunk diameter of HLB-affected trees. When trees are affected by HLB, they require higher Mn concentrations than healthy trees (NHLB), for similar physiological functions and possibly, to combat biotic stress. HLB-affected trees that received 22.4-kg Mn·ha^{-1}, which is about 4-times the UF/IFAS recommendation rate presented no visual signs of toxicity.

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Performance of HLB-affected Trees to Soil Macro- and Micronutrient Applications

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The huanglongbing (HLB) causative agent, *Candidatus* Liberibacter asiaticus, lowers tree performance by reducing water and nutrient uptake as a result of root loss. HLB-affected trees have a fibrous root loss of about 30 to 80%, which increases as HLB symptoms develop in the canopy. Investigating optimal nutrient concentrations in citrus roots thus improves our understanding of HLB dynamics concerning root nutrition and fertilizer application methods. This study sought to evaluate nutrient uptake of HLB-affected orange trees via soil fertilizer applications for 5- to 6-year-old *Citrus sinensis* 'Valencia' orange trees on Swingle rootstock at Ridge and Flatwoods sites. Macronutrients and micronutrients were applied at varying fertilization rates of standard fertilization via fertigation according to the University of Florida Institute of Food and Agricultural Science guidelines. For macronutrients, the rates were: a) standard fertilization + 40 lb/acre Ca + 40 lb/acre Mg + 220 lb/acre K and b) standard fertilization + 90 lb/acre Ca + 90 lb/acre Mg + 440 lb/acre K. For micronutrients, the rates were: a) standard fertilization + 5 lb/acre Fe, 5 lb/acre Mn, 5 lb/acre Zn+ 1 lb/acre B; b) standard fertilization + 10 lb/acre Fe, 10 lb/acre Mn, 10 lb/acre Zn + 2 lb/acre B; and c) standard fertilization + 20 lb/acre Fe, 20 lb/acre Mn + 20 lb/acre Zn + 4 lb/acre B. Soil and leaf samples were collected for nutrient concentration analysis in Spring and Fall 2019 and Summer 2020. No significant differences among treatments ($P < 0.05$) were observed for tissue and soil nutrient concentrations due to nutrient interactions. Fruit yield between the 2019 and 2020 harvest seasons increased with increased nutrient availability. Therefore, at higher fertilization rates of (standard fertilization + 40 lb/acre Ca + 40 lb/acre Mg + 220 lb/acre K + 20 lb/acre Fe, 20 lb/acre Mn + 20 lb/acre Zn + 4 lb/acre B), HLB-affected trees showed increased nutrient uptake, improving overall tree performance.

Florida citrus production has been on the decline for the past two decades, with orange production declining by 72%, from about 8 to 2 billion tons from 2007–2008 to 2017–2018 (National Agricultural Statistics Service, 2020). Citrus production area in Florida has also declined from over 750,000 acres in 2000 to approximately 392,515 acres in 2019 (National Agricultural Statistics Service, 2020). The decline in citrus production is mainly due to citrus greening and damages from the Hurricane Irma in 2017 (Dala-Paula et al., 2019).

Citrus greening (also known as huanglongbing or HLB), is a disease caused by a bacterium *Candidatus* Liberibacter asiaticus (C*Las*), which lowers tree performance due to reduced uptake and accumulation of water and nutrients (Kadyampakeni et al., 2014). The decline in uptake and accumulation of water and nutrients is due to an alteration of the plant's photosynthesis mechanism, root length density and the vascular system (Graham et al., 2013). Symptoms of HLB can be detected on several parts of the plant, from roots to leaves as well as increased acidity and bitterness of the fruit, thus changing the chemical and sensory characteristics of the fruit (Bassanezi et al., 2009; Bové, 2006). HLB-affected trees have a reduced canopy, leaves show chlorotic patterns and fruit size is reduced. The fruit contains small, brownish, aborted seeds that can be seen when the orange fruit is sectioned perpendicularly to the fruit axis. The presence of C*Las* pathogen in a plant causes the fruits to drop prematurely causing a 30 to 100% yield reduction, resulting in fruit losses of approximately $150 million annually (Gottwald et al., 2007).

Management strategies for HLB-affected trees include preventing the spread of infection by vector control and eliminating affected trees, while management of affected trees include pH regulation and foliar spray of readily absorbable nutrients and phytohormones to improve nutrient uptake (Dala-Paula et al., 2019). HLB-affected trees have a poorly developed and damaged root system due to fibrous root density loss of about 30 to 50%, which increases as HLB symptoms develop in the canopy. Thus, there is reduced nutrient uptake by the plants (Johnson and Graham, 2015).

The development of a proper nutrition program for citrus trees is important as it provides the essential elements required by the trees for maintenance, improved yield, and fruit quality (Aular et al., 2017). For green plants to function and grow well, 17 elements are essential, and among these, oxygen (O), carbon (C), and hydrogen (H) are freely abundant in nature (Havlin et al., 2014). The 14 other mineral elements are divided into macronutrients and micronutrients. Macronutrients are elements that a plant requires in large quantities while micronutrients are required in small quantities. Macronutrients include nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), and micronutrients include iron (Fe), zinc (Zn),

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manganese (Mn), boron (B), copper (Cu), molybdenum (Mo), nickel (Ni), and chlorine (Cl) (Havlin et al., 2014; Timilsena et al., 2014; Barker and Pilbeam, 2015; Zekri and Obreza, 2016). When an essential element is deficient, tree performance declines. Nutrient deficiencies result in distinct symptoms that can be exhibited in the leaves, twigs, and fruits while an excessive amount of an essential element can lead to toxicity, which hinders tree performance (Obreza and Morgan, 2008).

Potassium is an important macronutrient that helps in sugar and starch metabolism and protein synthesis (Abbas and Fares, 2009). Potassium is essential in citrus production because it helps improve fruit size, fruit number and fruit quality (Aly et al., 2015). K deficiency in citrus causes slow vegetative growth, which leads to reduced fruit number, thinning of foliage, fruit drop, and fruit splitting (Ritenour et al., 2003; Obreza and Morgan, 2008; Zekri and Obreza, 2016). Excess K content causes a decrease in juice quality because of a decrease in fruit total soluble solids, juice content, juice color, and sugar to acid ratio of the citrus (Ritenour et al., 2003). Sources of K in intensive citrus production systems in Florida are potassium chloride (KCl), potassium sulfate and potassium nitrate, with KCl having the greatest consumption in the US (Havlin et al., 2014).

Calcium is an essential nutrient in citrus production that helps to strengthen the cell wall structure and aids in root development and functioning (Crowley, 2012; Havlin et al, 2014). An adequate supply of Ca is essential in promoting tree growth and fruit development (Zekri and Obreza, 2016). An insufficient supply of Ca causes poor nutrient and water uptake due to low carbohydrate content in the roots, resulting in reduced plant growth and fruit yield (Havlin, et al, 2014; Zekri and Obreza, 2016).

Iron is an essential element in plant nutrition because of its vital role in nitrogen fixation (Srivastava, 2013). Iron availability is affected by phosphorus heavy metals. High P content and accumulation of heavy metals such as Cu cause Fe deficiency in the soil. Fe deficiency can also be induced by Zn and Mn deficiencies (Zekri and Obreza, 2016).

Zinc is important for the formation of auxins that promote growth in plants, the formation of chlorophyll, plant carbon metabolism and improving water uptake by plants (Hansch and Mendel 2009). Thus, an inadequate supply of Zn results in decreased plant growth, stress tolerance and chlorophyll synthesis (Kawachi et al. 2009). Thus, Zn fertilization is vital because it increases the availability of the nutrient in the soil and the content in the orange trees (Hippler, 2015).

Currently, there are no clear guidelines for determining optimal nutrient concentrations in citrus roots to understand the relationship between root nutrition and HLB-affected trees for improved management strategies (Morgan et al., 2006; Eissenstat, 1991; Castle and Krezdorn, 1975). Therefore, investigating optimal nutrient concentrations in citrus roots would help to improve our understanding of the dynamics of HLB concerning root nutrition and fertilizer application methods, most importantly fertigation and soil application. This study was conducted to evaluate nutrient uptake of HLB-affected orange trees because of differential macro- and micronutrient fertilization. The specific objectives were: 1) to determine optimal nutrient concentrations in the soil and leaves for orange trees and 2) compare soil fertilization rates to identify optimal fertilizer rate for nutrient uptake into both underground and above ground components. The study hypothesized that higher soil nutrient, and fertilization rates would result in optimal nutrient concentrations for improved tree health in HLB-affected orange trees and higher rates of micronutrient fertilization guidelines would improve root health of HLB affected orange trees, thereby improving the overall performance of the orange trees.

Materials and Methods

Site description. The study was conducted at the Citrus Research and Education Center (CREC), Lake Alfred, FL. (28°06'28.6"N; 81°41'07.8"W) and on a Flatwoods site near Clewiston, FL. (N 26°44'20.851"; W–81°4'54.568") to determine optimal fertigation and fertilization schemes. The two sites have 5- to 6-year-old *Citrus sinensis* 'Valencia' orange trees on Swingle rootstock. The Ridge soils at CREC site are Entisols classified as hyperthermic, uncoated lamellic quartzipsamments family (United States Department of Agriculture: Natural Resources Conservation Service, 2013). These soils are excessively drained and formed from eolian deposits and sandy marine deposits. The slope of the Ridge soils is 0 to 5% (United States Department of Agriculture: Natural Resources Conservation Service, 2019). Soils at the southwest Flatwoods site are Entisols of the siliceous, hyperthermic family of Mollic Psammaquents. The soils are poorly drained, rapidly permeable soils that are formed in sandy marine sediment underlain by limestone. The slope of these soils is 0 to 2% (United States Department of Agriculture: Soil Conservation Service, 1990). The Ridge soils have a high density of trees of about 1359 trees/ha while the Flatwoods soils of southwest Florida have a lower tree density of about 716 trees/ha.

Experimental design. The experimental design was a randomized complete block factorial design with an evaluation of macronutrients K and Ca at: a) 220 lb/acre K and 40 lb/acre Ca (1× macronutrients) and b) 440 lb/acre K and 90 lb/acre Ca (2× macronutrients); and micronutrients (Zn and Fe) at: a) 5 lb/acre (1× micronutrients), b)10 lb/acre (2× micronutrients), and c) 20 lb/acre (4× of micronutrients) of the current University of Florida Institute of Food and Agricultural Science (UF IFAS) fertilization guidelines (Obreza and Morgan, 2008; Morgan and Kadyampakeni, 2020). Macronutrients and micronutrients were applied three times per year on the soil. Each plot had 10 trees where the middle 8 trees were the experimental unit. All treatments were replicated 6 times.

The treatments were as follows:

- Control with standard fertilization via fertigation of N, P, S, Mo, and Cu fertilization according to UF IFAS guidelines. No extra K, Mg, Ca, Mn, Fe, B, and Zn.
- Standard fertilization + 1X macronutrient (MA) + 1× micronutrient (MI) (soil applied).
- Standard fertilization + 1× MA + 2× MI (soil applied).
- Standard fertilization + 1× MA + 4× MI (soil applied).
- Standard fertilization + 2× MA + 1× MI (soil applied).
- Standard fertilization + 2× MA + 2× MI (soil applied).
- Standard fertilization + 2× MA + 4× MI (soil applied).

Sampling methods. To determine optimal nutrient concentrations in the soil and leaves for orange trees, leaf and soil nutrient concentrations were evaluated in May and Nov. 2019 and July 2020. About 20 mature leaves were collected at each time point in in the northwest, southwest, northeast, and southeast directions with reference to the sampled orange tree. Soil samples were collected at a depth of 0–15 cm. Soil and leaf samples were processed for nutrient content analysis.

Data analysis. Data analyses to evaluate if there are treatment, synergistic or interaction effects among macro- and micronutrient application rates and relationships between fertilizer application rates and nutrient content were done using R version 4.0.2 (R Core Team, 2013). The variates evaluated were plant tissue and soil concentrations of K, Ca, Fe, and Zn. Analysis of variance (ANOVA) was done to compare treatments once the ANOVA assumptions such as normality, homogeneity of variance and uniform distribution of the data, were met. Tukey's Honestly Significant Difference (HSD) test was conducted to evaluate the means that were significantly different from the others based on the variates under evaluation.

Results

Plant tissue nutrient concentrations. There was a gradual increase in K concentrations from May 2019 to July 2020 (Table 1). No significant differences were obtained in K concentrations of the leaf tissues among treatments (ANOVA, $P = 0.05$). Trees that received Treatment 5 showed the highest K concentration in July 2020 and Treatment 6 had the lowest leaf K concentration (Table 1). Leaf Ca concentrations decreased from May 2019 to July 2020. There were no significant differences in Ca concentrations among treatments throughout the period of study. Treatment 7 had the greatest leaf Ca concentrations, followed by Treatments 1, 4, and 6, while Treatment 5 had the least leaf Ca concentration. The results of the study showed that for all treatments, there was an increase in Zn concentrations at Flatwoods site in July 2020 as compared to May 2019 when the project had just started (Table 2). However, the results from these trials show no significant differences in Zn plant tissue concentrations among all the treatments ($P > 0.05$). Zinc concentrations in plant tissue ranged between 40.17 ± 8.42 ppm and 48.67 ± 4.80 ppm. Iron exhibited a different trend in its concentrations throughout the period of study, which show a reduction in Fe concentrations in Nov. 2019 as compared to May 2019. Nevertheless, there was an increase in the Fe concentrations in plant tissues in July 2020. The increase in Fe concentrations in plant tissues did not differ significantly among the treatments ($P > 0.05$). Treatment 4 had the highest Fe concentration while Treatment 2 had the least Fe concentration.

Potassium concentrations decreased sharply in Nov. 2019 at the Ridge site, and gradually increased in July 2020 for all treatments (Table 3). No significant differences among treatments ($P > 0.05$) were observed. There was an increase in Ca concentrations at Ridge site in Nov. 2019 as compared to May 2019. However, leaf Ca concentrations decreased in July 2020. There were no significant differences in leaf Ca concentrations among treatments. At the end of the trial, Treatment 7 had the greatest leaf Ca concentration while Treatment 2 had the lowest Ca concentration. Results further showed that for all treatments, there was an increase in Zn concentrations at Ridge site in July 2020 in comparison to May 2019 (Table 4). However, the results obtained did not have significant differences among all the treatments ($P > 0.05$). Concentrations of Zn ranged between 28.67 ± 4.23 ppm and 33.67 ± 4.72 ppm. Leaf tissue concentrations of Fe decreased greatly in July 2020 as compared to May 2019. In July 2020, the control (Treatment 1) had the highest Fe concentration of 45.50 ± 2.51 ppm while Treatment 6 had the lowest Fe concentration of 38.00 ± 4.82 ppm.

Soil nutrient concentrations. A trend similar to that of leaf tissue nutrient concentrations was observed for nutrient concentrations in the soil. Soil K concentrations at the Flatwoods site increased from May 2019 to Nov. 2019 (Table 5). There were no significant differences among treatments for soil K concentrations. The concentration of K in the soil ranged between 54 ± 21 mg $kg^{-1}$and 83 ± 36 mg kg^{-1}. Soil Ca concentrations decreased between May 2019 and Nov. 2019. The concentration of Ca

Table 1. Potassium (K) and calcium (Ca) uptake in the leaves of *Citrus sinensis* 'Valencia' orange trees as a function of differential fertilizer application rates at a Flatwoods site in central Florida.

	K			Ca		
	%					
Treatment	May 2019	Nov. 2019	July 2020	May 2019	Nov. 2019	July 2020
1[z]	1.36 ± 0.36[y]	1.61 ± 0.10	1.76 ± 0.10	4.29 ± 0.50	4.04 ± 0.42	3.97 ± 0.59
2[x]	1.38 ± 0.27	1.63 ± 0.18	1.78 ± 0.11	4.07 ± 0.38	3.94 ± 0.35	3.87 ± 0.26
3[w]	1.49 ± 0.11	1.46 ± 0.10	1.76 ± 0.09	4.11 ± 0.32	4.14 ± 0.46	3.96 ± 0.49
4[v]	1.44 ± 0.33	1.63 ± 0.12	1.82 ± 0.09	4.08 ± 0.40	3.81 ± 0.19	3.94 ± 0.30
5[u]	1.66 ± 0.09	1.49 ± 0.12	1.85 ± 0.11	3.84 ± 0.22	4.09 ± 0.55	3.72 ± 0.22
6[t]	1.51 ± 0.17	1.54 ± 0.19	1.75 ± 0.15	4.19 ± 0.41	3.88 ± 0.47	3.72 ± 0.26
7[s]	1.55 ± 0.14	1.62 ± 0.15	1.77 ± 0.08	3.97 ± 0.34	3.98 ± 0.44	3.98 ± 0.13
P-value	0.34 ns	0.22 ns	0.58 ns	0.51 ns	0.82 ns	0.71 ns

[z]Control with standard fertilization via fertigation of N, P, S, Mo, Cu fertilization according to UF/IFAS guidelines. No extra K, Mg, Ca, Mn, Fe, B, and Zn.

[y] Means ± SD followed by different lowercase letters which are significantly different at $P < 0.05$ by Tukey's HSD test.

[x]Standard fertilization + 1× macronutrient (MA) + 1× micronutrient (MI) (soil applied).

[w]Standard fertilization + 1× MA + 2× MI (soil applied).

[v]Standard fertilization + 1× MA + 4× MI (soil applied).

[u]Standard fertilization + 2× MA + 1× MI (soil applied).

[t]Standard fertilization + 2× MA + 2× MI (soil applied).

[s]Standard fertilization + 2× MA + 4× MI (soil applied).

ns = nonsignificant at $P < 0.05$ by Tukey's HSD test. 1× and 2× MA refer to UF/IFAS recommendation of the 45 and 90 kg/ha (40 and 90 lb/acre) of macronutrients Ca and Mg and 220 and 440 lb/acre of K. 1×, 2× and 4× MI refer to 5.6, 11.2 and 22.4 kg/ha (or 5, 10, and 20 lb/acre) of micronutrients Fe, Mn and Zn, and 1-, 2-, and 4-lb/acre of B per year.

ranged between 793 ± 224 mg kg^{-1} and 1335 ± 832 mg kg^{-1}. No significant differences were observed among treatments for Ca concentrations in the soil. Soil Zn concentration increased between May 2019 and Nov. 2019 (Table 6). No significant differences among treatments were observed for Zn concentrations. Treatment 7 had the highest Zn concentration of 38 ±23 mg kg^{-1} while Treatment 2 had the least Zn concentration of 19±7 mg kg^{-1}. Generally, there was a decrease in Fe concentration from May 2019 to Nov. 2019. Soil Fe concentrations ranged between 126 ± 24 mg kg^{-1} and 147 ± 26 mg kg^{-1} (Table 6).

Similarly, soil K concentrations at Ridge site decreased from May 2019 to July 2020 (Table 7). The difference in K concentrations among treatments was not significant in July 2020. Treatment 2 had the highest K concentration of 32 ± 11 mg kg^{-1} while Treatment 1

Table 2. Zinc (Zn) and iron (Fe) uptake in the leaves of *Citrus sinensis* 'Valencia' orange trees as a function of differential fertilizer application rates at a Flatwoods site in central Florida.

	Zn			Fe		
	ppm					
Treatment	May 2019	Nov. 2019	July 2020	May 2019	Nov. 2019	July 2020
1[z]	22.54 ± 3.68[y]	29.00 ± 4.47	44.50 ± 9.85	61.77 ± 6.26	58.67 ± 6.86	66.30 ± 14.10
2[x]	22.47 ± 3.11	28.67 ± 2.58	40.17 ± 8.42	60.11 ± 7.26	54.33 ± 2.73	64.67 ± 13.29
3[w]	26.06 ± 3.43	28.00 ± 2.90	46.33 ± 9.29	65.06 ± 11.38	57.50 ± 6.22	71.50 ± 9.40
4[v]	23.73 ± 2.02	28.83 ± 4.92	48.67 ± 4.80	66.25 ± 4.26	63.50 ± 17.21	72.50 ± 9.61
5[u]	26.48 ± 2.79	27.00 ± 2.00	44.50 ± 4.89	63.11 ± 10.48	51.17 ± 4.49	63.67 ± 6.59
6[t]	25.06 ± 4.95	28.33 ± 5.16	42.50 ± 6.09	61.64 ± 3.83	58.33 ± 10.56	70.17 ± 12.37
7[s]	23.51 ± 3.36	27.83 ± 2.48	42.83 ± 9.87	63.44 ± 4.46	57.50 ± 3.02	71.33 ± 14.22
P-value	0.27 ns	0.97 ns	0.63 ns	0.81 ns	0.35 ns	0.75 ns

[z]Control with standard fertilization via fertigation of N, P, S, Mo, and Cu fertilization according to UF/IFAS guidelines. No extra K, Mg, Ca, Mn, Fe, B, and Zn.

[y]Means ± SD followed by different lowercase letters which are significantly different at $P < 0.05$ by Tukey's HSD test.

[x]Standard fertilization + 1× macronutrient (MA) + 1× micronutrient (MI) (soil applied).

[w]Standard fertilization + 1× MA + 2× MI (soil applied).

[v]Standard fertilization + 1× MA + 4× MI (soil applied).

[u]Standard fertilization + 2× MA + 1× MI (soil applied).

[t]Standard fertilization + 2× MA + 2× MI (soil applied).

[s]Standard fertilization + 2× MA + 4× MI (soil applied).

ns = nonsignificant at $P < 0.05$ by Tukey's HSD test. 1× and 2× MA refer to UF/IFAS recommendation of the 45 and 90 kg/ha (40 and 90 lb/acre) of macronutrients Ca and Mg and 220 and 440 lb/acre of K. 1×, 2v, and 4v MI refer to 5.6, 11.2, and 22.4 kg/ha (or 5, 10. and 20 lb/acre) of micronutrients Fe, Mn, and Zn, and 1-, 2- and 4-lb/acre of B per year.

Table 3. Potassium (K) and calcium (Ca) uptake in the leaves of *Citrus sinensis* 'Valencia' orange trees as a function of differential fertilizer application rates at a Ridge site in central Florida.

	K			CA		
	ppm					
Treatment	May 2019	Nov. 2019	July 2020	May 2019	Nov. 2019	July 2020
1[z]	1.71 ± 0.12[y]	1.30 ± 0.11	1.31 ± 0.1	3.56 ± 0.10	3.85 ± 0.27	3.16 ± 0.12
2[x]	1.67 ± 0.10	1.21 ± 0.13	1.50 ± 0.07	3.41 ± 0.22	4.26 ± 0.40	3.08 ± 0.20
3[w]	1.69 ± 0.11	1.24 ± 0.28	1.39 ± 0.19	3.28 ± 0.25	4.21 ± 0.33	3.23 ± 0.22
4[v]	1.66 ± 0.11	1.30 ± 0.25	1.48 ± 0.16	3.30 ± 0.24	3.97 ± 0.33	3.09 ± 0.26
5[u]	1.69 ± 0.09	1.21 ± 0.09	1.39 ± 0.31	3.50 ± 0.24	4.14 ± 0.34	3.16 ± 0.1
6[t]	1.72 ± 0.07	1.26 ± 0.14	1.40 ± 0.23	3.25 ± 0.33	4.03 ± 0.25	3.24 ± 0.24
7[s]	1.78 ± 0.14	1.20 ± 0.18	1.39 ± 0.24	3.25 ± 0.26	4.35 ± 0.30	3.27 ± 0.27
P-value	0.62 ns	0.92 ns	0.72 ns	0.17 ns	0.13 ns	0.64 ns

[z]Control with standard fertilization via fertigation of N, P, S, Mo, Cu fertilization according to UF/IFAS guidelines. No extra K, Mg, Ca, Mn, Fe, B, and Zn.

[y]Means ± SD followed by different lowercase letters which are significantly different at $P < 0.05$ by Tukey's HSD test

[x]Standard fertilization + 1× macronutrient (MA) + 1× micronutrient (MI) (soil applied).

[w]Standard fertilization + 1× MA + 2× MI (soil applied).

[v]Standard fertilization + 1× MA + 4× MI (soil applied).

[u]Standard fertilization + 2× MA + 1× MI (soil applied).

[t]Standard fertilization + 2× MA + 2× MI (soil applied).

[s]Standard fertilization + 2× MA + 4× MI (soil applied).

ns = nonsignificant at $P < 0.05$ by Tukey's HSD test. 1× and 2× MA refer to UF/IFAS recommendation of the 45 and 90 kg/ha (40 and 90 lb/acre) of macronutrients Ca and Mg and 220 and 440 lb/per acre of K. 1×, 2× and 4× MI refer to 5.6, 11.2 and 22.4 kg/ha (or 5, 10, and 20 lb/acre) of micronutrients Fe, Mn, and Zn, and 1-, 2-, and 4-lb/acre of B per year.

had the least K concentration of 29 ± 12 mg kg^{-1}. There was a reduction in Ca concentration from May 2019 to July 2020. Calcium concentration in the soil ranged between 603 ± 82 mg kg^{-1} and 720 ± 6 mg kg^{-1}. Zinc concentration in the soil increased over time, from May 2019 to July 2020. No significant differences among treatments were observed for Zn concentrations; with Treatment 7 having the greatest Zn concentration of 60 ± 19 mg kg^{-1} while Treatment 5 had the least K concentration of 43 ± 8 mg kg^{-1} (Table 8). There was a reduction in Fe concentration in the soil from May 2019 to July 2020. Concentration of Fe ranged between 124 ± 20 mg kg-1 and 144 ± 15 mg kg^{-1}.

Fruit yield. There was an increase in fruit yield between 2019 and 2020 at the Ridge site ranging between 26.3 % and 55.8% (Table 9). Treatment 2 had the greatest yield in 2020 of 12.2 ± 1.5 t/ha while Treatment 5 had the least yield of 9.6 ± 2.9 t/ha. There was no comparison of yield data for Flatwoods

Table 4. Zinc (Zn) and Iron (Fe) uptake in the leaves of *Citrus sinensis* 'Valencia' orange trees as a function of differential fertilizer application rates at a Ridge site in central Florida.

	Zn			Fe		
	ppm					
Treatment	May 2019	Nov. 2019	July-2020	May 2019	Nov. 2019	July 2020
1[z]	19.41 ± 4.26[y]	14.17 ± 2.56	30.67 ± 1.86	75.04 ± 6.75	59.67 ± 24.95	45.50 ± 2.51
2[x]	21.22 ± 2.00	14.50 ± 1.76	28.83 ± 6.91	84.80 ± 6.22	45.33 ± 4.89	39.00 ± 2.28
3[w]	20.24 ± 2.56	14.33 ± 3.56	28.67 ± 4.23	72.51 ± 9.43	62.00 ± 18.22	41.00 ± 4.94
4[v]	19.64 ± 0.94	13.83 ± 1.83	33.67 ± 4.72	77.14 ± 7.99	45.17 ± 6.65	43.00 ± 4.52
5[u]	20.37 ± 3.32	14.33 ± 1.03	29.50 ± 4.37	80.86 ± 10.99	48.17 ± 4.31	39.50 ± 6.53
6[t]	21.36 ± 2.17	15.17 ± 3.19	29.83 ± 4.88	79.67 ± 11.18	48.33 ± 6.35	38.00 ± 4.82
7[s]	20.80 ± 1.76	14.17 ± 1.83	28.83 ± 4.45	77.43 ± 12.80	57.33 ± 14.21	45.33 ± 7.55
P-value	0.82 ns	0.98 ns	0.54 ns	0.41 ns	0.16 ns	0.07 ns

[z]Control with standard fertilization via fertigation of N, P, S, Mo, Cu fertilization according to UF/IFAS guidelines. No extra K, Mg, Ca, Mn, Fe, B, and Zn.
[y]Means ± SD followed by different lowercase letters which are significantly different at $P < 0.05$ by Tukey's HSD test
[x]Standard fertilization + 1× macronutrient (MA) + 1× micronutrient (MI) (soil applied).
[w]Standard fertilization + 1× MA + 2× MI (soil applied).
[v]Standard fertilization + 1× MA + 4× MI (soil applied).
[u]Standard fertilization + 2× MA + 1× MI (soil applied)
[t]Standard fertilization + 2× MA + 2× MI (soil applied).
[s]Standard fertilization + 2× MA + 4× MI (soil applied).
ns = nonsignificant at $P < 0.05$ by Tukey's HSD test. 1× and 2× MA refer to UF/IFAS recommendation of the 45 and 90 kg/ha (40 and 90 lb/acre) of macronutrients Ca and Mg and 220 and 440 lb/acre of K. 1×, 2×, and 4× MI refer to 5.6, 11.2, and 22.4 kg/ha (or 5, 10, and 20 lb/acre) of micronutrients Fe, Mn and Zn, and 1-, 2-, and 4-lb/per acre of B per year.

Table 5. Soil potassium (K) and calcium (Ca) concentrations as a function of differential fertilizer application rates at a Flatwoods site in central Florida.

	K		Ca	
	mg/kg			
Treatment	May 2019	Nov. 2019	May 2019	Nov. 2019
1[z]	47.00 ± 11.82[y]	59.13 ± 6.02	2334 ± 2024	1335 ± 832
2[x]	49.67 ± 14.46	66.17 ± 39.14	2280 ± 3392	1309 ± 805
3[w]	56.08 ± 27.92	65.25 ± 39.13	1147 ± 779	1215 ± 585
4[v]	66.50 ± 26.59	58.13 ± 17.68	1505 ± 1231	793 ± 224
5[u]	47.67 ± 24.93	67.96 ± 47.67	2131 ± 2067	1096 ± 565
6[t]	42.50 ± 13.77	54.08 ± 20.97	1104 ± 769	638 ± 336
7[s]	43.75 ± 15.33	82.63 ± 35.71	1350 ± 949	837 ± 416
P-value	0.43 ns	0.81 ns	0.79 ns	0.25 ns

[z]Control with standard fertilization via fertigation of N, P, S, Mo, and Cu fertilization according to UF/IFAS guidelines. No extra K, Mg, Ca, Mn, Fe, B, and Zn.
[y]Means ± SD followed by different lowercase letters which are significantly different at $P < 0.05$ by Tukey's HSD test.
[x]Standard fertilization + 1× macronutrient (MA) + 1× micronutrient (MI) (soil applied).
[w]Standard fertilization + 1× MA + 2× MI (soil applied).
[v]Standard fertilization + 1× MA + 4× MI (soil applied).
[u]Standard fertilization + 2× MA + 1× MI (soil applied).
[t]Standard fertilization + 2× MA + 2× MI (soil applied).
[s]Standard fertilization + 2× MA + 4× MI (soil applied).
ns = nonsignificant at $P < 0.05$ by Tukey's HSD test. 1× and 2× MA refer to UF/IFAS recommendation of the 45 and 90 kg/ha (40 and 90 lb/acre) of macronutrients Ca and Mg and 220 and 440 lb/acre of K. 1×, 2×, and 4× MI refer to 5.6, 11.2 and 22.4 kg/ha (or 5, 10, and 20 lb/acre) of micronutrients Fe, Mn and Zn, and 1-, 2- and 4-lb/acre of B per year.

site since no data collection for 2020 was done due to the COVID-19 lockdown.

Discussion

Tissue nutrient concentrations. The trend in calcium foliar concentrations can be due to interactions with K concentrations in the soil. Increased K concentrations in the soil cause a decrease in root uptake of Ca and Mg (Jakobsen, 1993). Thus, for the Flatwoods site, there was an increase in K concentrations throughout the period of study, which had an antagonistic effect on Ca, resulting in reduced Ca concentrations. Similarly, for the Ridge site, Ca concentrations were relatively high, which resulted in reduced K concentrations in leaf tissues.

Calcium and Fe also have an antagonistic effect on Zn availability and translocation within the plant. At higher fertilization rates, there was an increase in Ca and Fe concentrations, which reduce the absorption of Zn by the roots and its translocation to the leaves (Prasad et al., 2016). Similar trends were also observed by Phuyal et. al (2020), whose study conducted on HLB-affected

Table 6. Soil zinc (Zn) and iron (Fe) concentrations as a function of differential fertilizer application rates at a Flatwoods site in central Florida.

	Zn		Fe	
	mg/kg			
Treatment	May 2019	Nov. 2019	May 2019	Nov. 2019
1[z]	14.68 ± 7.92[y]	32.75 ± 32.51	127.92 ± 41.03	138.92 ± 21.14
2[x]	13.31 ± 8.39	19.21 ± 7.19	136.58 ± 54.45	125.50 ± 24.24
3[w]	20.78 ± 16.09	22.40 ± 10.18	169.83 ± 25.86	157.54 ± 18.39
4[v]	17.32 ± 10.78	29.27 ± 13.68	126.17 ± 34.28	146.96 ± 25.95
5[u]	20.71 ± 6.03	28.80 ± 7.03	151.33 ± 46.17	131.75 ± 12.99
6[t]	11.26 ± 7.27	25.92 ± 7.03	150.17 ± 18.09	134.21 ± 36.82
7[s]	14.93 ± 7.69	37.45 ± 22.53	155.83 ± 50.31	132.29 ± 27.76
P-value	0.54 ns	0.58 ns	0.49 ns	0.36 ns

[z]Control with standard fertilization via fertigation of N, P, S, Mo, and Cu fertilization according to UF/IFAS guidelines. No extra K, Mg, Ca, Mn, Fe, B, and Zn.
[y]Means ± SD followed by different lowercase letters which are significantly different at $P < 0.05$ by Tukey's HSD test.
[x]Standard fertilization + 1× macronutrient (MA) + 1× micronutrient (MI) (soil applied).
[w]Standard fertilization + 1× MA + 2× MI (soil applied).
[v]Standard fertilization + 1× MA + 4× MI (soil applied).
[u]Standard fertilization + 2× MA + 1× MI (soil applied).
[t]Standard fertilization + 2× MA + 2× MI (soil applied).
[s]Standard fertilization + 2× MA + 4× MI (soil applied).
ns = nonsignificant at $P < 0.05$ by Tukey's HSD test. 1× and 2× MA refer to UF/IFAS recommendation of the 45 and 90 kg/ha (40 and 90 lb/acre) of macronutrients Ca and Mg and 220 and 440 lb/acre of K. 1×, 2× and 4× MI refer to 5.6, 11.2, and 22.4 kg/ha (or 5, 10, and 20 lb/acre) of micronutrients Fe, Mn and Zn, and 1-, 2-, and 4-lb/per acre of B per year.

Table 7. Soil potassium (K) and calcium (Ca) concentrations as a function of differential fertilizer application rates at a Ridge site in central Florida.

	K			Ca		
	mg/kg					
Treatment	May 2019	Nov. 2019	July 2020	May 2019	Nov. 2019	July 2020
1[z]	73.08 ± 14.94[y]	40.46 ± 6.57 b	24.75 ± 5.15	760.33 ± 102.29	596.92 ± 82.85	720.42 ± 96.12
2[x]	62.25 ± 9.50	53.54 ± 18.00 ab	31.67 ± 10.87	786.50 ± 195.10	429.75 ± 141.64	616.17 ± 63.66
3[w]	69.58 ± 10.22	49.75 ± 15.88 b	29.92 ± 9.47	811.83 ± 131.26	519.42 ± 109.06	811.08 ± 301.29
4[v]	65.33 ± 13.63	61.04 ± 36.26 ab	29.67 ± 6.66	752.50 ± 263.53	452.50 ± 65.86	602.58 ± 82.36
5[u]	66.75 ± 15.01	51.88 ± 21.79 ab	30.42 ± 16.45	866.25 ± 97.13	486.63 ± 191.64	626.25 ± 98.58
6[t]	68.25 ± 10.12	117.67 ± 74.72 a	28.67 ± 11.59	765.17 ± 156.75	527.25 ± 67.80	681.17 ± 150.07
7[s]	71.92 ± 9.33	78.58 ± 40.49 ab	30.08 ± 6.51	880.75 ± 177.85	564.83 ± 87.21	711.33 ± 131.63
P-value	0.74 ns	0.02*	0.94 ns	0.76 ns	0.17 ns	0.17 ns

[z]Control with standard fertilization via fertigation of N, P, S, Mo, Cu fertilization according to UF/IFAS guidelines. No extra K, Mg, Ca, Mn, Fe, B, and Zn.
[y]Means ± SD followed by different lowercase letters which are significantly different at $P < 0.05$ by Tukey's HSD test.
[x]Standard fertilization + 1× macronutrient (MA) + 1× micronutrient (MI) (soil applied).
[w]Standard fertilization + 1× MA + 2× MI (soil applied).
[v]Standard fertilization + 1× MA + 4× MI (soil applied).
[u]Standard fertilization + 2× MA + 1× MI (soil applied).
[t]Standard fertilization + 2× MA + 2× MI (soil applied).
[s]Standard fertilization + 2× MA + 4× MI (soil applied).
ns, * = nonsignificant or significant at $P < 0.05$ by Tukey's HSD test, respectively. 1× and 2× MA refer to UF/IFAS recommendation of the 45 and 90 kg/ha (40 and 90 lb/acre) of macronutrients Ca and Mg and 220 and 440 lb/acre of K. 1×, 2×, and 4× MI refer to 5.6, 11.2 and 22.4 kg/ha (or 5, 10, and 20 lb.acre) of micronutrients Fe, Mn and Zn, and 1-, 2-, and 4-lb/acre of B per year.

Table 8. Soil zinc (Zn) and iron (Fe) concentrations as a function of differential fertilizer application rates at a Ridge site in central Florida.

	Zn			Fe		
	mg/kg					
Treatment	May 2019	Nov. 2019	July 2020	May 2019	Nov. 2019	July 2020
1[z]	43.63 ± 11.40[y]	40.45 ± 24.73	48.38 ± 7.73	141.42 ± 11.26	133.29 ± 10.61	129.75 ± 16.20
2[x]	42.43 ± 12.92	27.47 ± 7.81	45.63 ± 14.71	136.25 ± 11.04	136.00 ± 22.84	143.92 ± 14.84
3[w]	45.52 ± 12.24	37.05 ± 14.94	55.60 ± 17.68	131.08 ± 15.18	132.46 ± 18.13	123.58 ± 19.99
4[v]	43.70 ± 16.04	59.74 ± 21.62	54.61 ± 10.26	133.67 ± 13.19	132.88 ± 9.51	137.50 ± 15.06
5[u]	38.89 ± 7.83	28.69 ± 14.29	42.59 ± 7.46	131.25 ± 16.01	136.67 ± 28.33	142.25 ± 9.03
6[t]	45.15 ± 13.33	48.30 ± 15.48	55.23 ± 17.70	138.58 ± 11.51	132.75 ± 10.78	136.25 ± 15.90
7[s]	48.77 ± 12.80	52.18 ± 35.00	59.46 ± 18.91	136.42 ± 13.16	125.17 ± 14.69	128.00 ± 23.80
P-value	0.91 ns	0.09 ns	0.63 ns	0.80 ns	0.95 ns	0.92 ns

[z]Control with standard fertilization via fertigation of N, P, S, Mo, and Cu fertilization according to UF/IFAS guidelines. No extra K, Mg, Ca, Mn, Fe, B, and Zn.

[y]Means ± SD followed by different lowercase letters are significantly different at $P < 0.05$ by Tukey's HSD test.

[x]Standard fertilization + 1× macronutrient (MA) + 1× micronutrient (MI) (soil applied).

[w]Standard fertilization + 1× MA + 2× MI (soil applied).

[v]Standard fertilization + 1× MA + 4× MI (soil applied).

[u]Standard fertilization + 2× MA + 1× MI (soil applied).

[t]Standard fertilization + 2× MA + 2× MI (soil applied).

[s]Standard fertilization + 2× MA + 4× MI (soil applied).

ns = nonsignificant at $P < 0.05$ by Tukey's HSD test. 1× and 2× MA refer to UF/IFAS recommendation of the 45 and 90 kg/ha (40 and 90 lb/acre) of macronutrients Ca and Mg and 220 and 440 lb/acre of K. 1×, 2× and 4× MI refer to 5.6, 11.2, and 22.4 kg/ha (or 5, 10, and 20 lb/acre) of micronutrients Fe, Mn and Zn, and 1-, 2- and 4-lb/acre of B per year.

Table 9. Fruit yield of *Citrus sinensis* 'Valencia' orange trees at Flatwoods and Ridge sites in central Florida as a function of differential fertilizer application rates (t/ha).

	Fruit yield (t/ha)		
	Ridge site		Flatwoods site
Treatment	2019	2020	2019
1[z]	7.5 ± 3.0[y]	11.5 ± 3.1	13.3 ± 1.5
2[x]	8.0 ± 2.3	12.2 ± 1.5	13.8 ± 1.7
3[w]	7.8 ± 2.4	11.6 ± 3.4	13.5 ± 1.8
4[v]	8.4 ± 3.5	13.0 ± 2.1	14.1 ± 2.7
5[u]	7.6 ± 2.0	9.6 ± 2.9	13.4 ± 2.7
6[t]	7.7 ± 2.6	12.0 ± 3.2	13.9 ± 3.3
7[s]	8.3 ± 2.3	10.7 ± 3.3	13.0 ± 1.3
P-value	0.995 ns	0.526 ns	0.984 ns

[z]Control with standard fertilization via fertigation of N, P, S, Mo, Cu fertilization according to UF/IFAS guidelines. No extra K, Mg, Ca, Mn, Fe, B, and Zn.

[y]Means ± SD followed by different lowercase letters are significantly different at $P < 0.05$ by Tukey's HSD test.

[x]Standard fertilization + 1× macronutrient (MA) + 1× micronutrient (MI) (soil applied).

[w]Standard fertilization + 1× MA + 2× MI (soil applied).

[v]Standard fertilization + 1× MA + 4× MI (soil applied).

[u]Standard fertilization + 2× MA + 1× MI (soil applied).

[t]Standard fertilization + 2× MA + 2× MI (soil applied).

[s]Standard fertilization + 2× MA + 4× MI (soil applied).

ns = nonsignificant at $P < 0.05$ by Tukey's HSD test respectively. 1× and 2× MA refer to UF/IFAS recommendation of the 45 and 90 kg/ha (40 and 90 lb/acre) of macronutrients Ca and Mg and 220 and 440 lb/acre of K. 1×, 2× and 4× MI refer to 5.6, 11.2, and 22.4 kg/ha (or 5, 10, and 20 lb/acre) of micronutrients Fe, Mn, and Zn, and 1-, 2- and 4-lb/acre of B per year.

'Ray Ruby' grapefruit showed an increase in K and Zn due to interactions with other nutrients.

Soil nutrient concentrations. Nutrient interactions played a huge role in the availability of nutrients for orange tree uptake. An antagonistic effect between K and Ca was observed. An increase in K concentration in the soil resulted in a decrease in Ca at both the Flatwoods and Ridge sites. Calcium also competes with zinc for adsorption sites on soil particles and root particles. Thus, with increased Zn concentrations in the soil, there will be fewer adsorption sites for Ca resulting in reduced Ca concentrations. Additionally, interaction effects were observed between Zn and Fe. Zinc negatively affects the absorption of Fe by plants (Prasad et.al. 2016). Thus, with the increased availability of Zn in the soil, there was a disruption in absorption of Fe by orange trees hence Fe concentration decreased in the soil at both the Flatwoods and Ridge sites.

Fruit yield. Generally, fruit yield increased at higher fertilization rates due to increased nutrient availability. Potassium availability increased with increased fertilization rate, thus enhancing functions such as fruit formation. These results are consistent with studies by Koo (1962), which showed an increase in fruit yield with increased K content while a decrease in yield was observed when K fertilizer was deficient in the treatments though this was done several years before the advent of HLB.

Conclusions

Nutrient availability for HLB-affected *Citrus sinensis* 'Valencia' orange trees is affected by interactions of nutrients in the soil. An increase in K concentration results in a decrease in Ca concentration while an increase in Zn concentration reduces Fe concentration in the soil for tree uptake. Optimum nutrient concentrations for K, Ca, Zn, and Fe in plant tissues of 1.9 ± 0.1 %, 4.0 ± 0.13%, 48.7 ± 4.8 ppm and 45.5 ± 2.5 ppm respectively, are

suggested. For soil nutrient content, optimum nutrient concentrations of 83 ± 36 mg·kg^{-1}, 38 ± 23 mg·kg^{-1} and 147 ± 26 mg·kg^{-1} are suggested for Ca, Zn, and Fe, respectively. Higher fertilizer application rates increase nutrient availability, particularly K, which subsequently results in increased yield of HLB-affected orange trees.

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Performance of Leaf Wetness Sensors for Applicability In Decision-support Systems for Management of Citrus, Blueberry, and Strawberry Diseases

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Disease Alert Systems (DAS) in the Agroclimate decision-support system provide site-specific information to aid citrus, blueberry, and strawberry growers to decide when a fungicide application would be required. All of these DAS use disease models based on temperature and leaf wetness (LW) duration data to predict when weather conditions are favorable for disease development and control measures are needed. Daily environmental data are obtained from the weather stations of the Florida Agricultural Weather Network (FAWN). Previous research has shown that the electrical resistance-based Campbell 237-L leaf wetness (LW) sensors provide reliable data. However, they require painting and in-situ calibration, which is not easily done by growers. Conversely, Decagon LW dielectric sensors come ready to use by the manufacturer, with pre-established thresholds for wet and dry conditions. However, their performance in the field is uncertain. We compared the LW estimations provided by Campbell 237-L and Decagon dielectric sensors installed in the same station in Plant City, Florida. We performed comparisons of every sensor combination using 15-minute observations and maximum daily LW duration. The sensors of the same manufacturer had high (> 0.90) Pearson's correlation coefficient (Pc), low (< 1.0) mean absolute error (MAE), and high *k* agreement indices (> 0.9), which indicate a strong correlation. However, when comparing Campbell and Decagon sensors, the precision was lower as indicated by Pc of approximately 0.8, MAE around 2.0 hours, and k-indices around 0.8. Nevertheless, the estimations MAE were within the acceptable range for DAS applicability. Decagon dielectric sensors could be used in the FAWN weather stations to provide reliable LW estimations.

Disease Alert Systems (DAS) intended for plant disease management routinely use leaf wetness (LW) as input for disease risk calculations. LW can be estimated by sensors specifically built for that purpose or by mathematical models that use commonly observed weather variables, most often relative humidity. There is a wide array of different LW sensors available in the market and no standardized way to measure LW in the field (Rowlandson et al. 2015). Two types of LW sensors are used in weather stations in the Florida Automated Weather Network (FAWN) that provide information to DAS — the Decagon LW dielectric sensor (Decagon Devices Inc., Pullman, WA) and the electrical resistance-based Campbell 237-L sensors (Campbell Scientific, Logan, UT). Each sensor has benefits and drawbacks in comparison to the other. For instance, the Decagon LW dielectric sensors have the advantage of being calibrated and painted by the manufacturer. The electrical resistance-based Campbell 237-L sensors provide reliable LW estimations (Sentelhas et al. 2004a) and have been functional for more than ten years after installation (Peres, personal observation). The drawback of Decagon dielectric sensors was their low durability. In previous trials, it was observed that older models were functional for approximately one to two years in Florida fields (Peres and Fraisse, personal observation). However, it is our understanding that Decagon has since made improvements to its sensors. The Campbell 237-L leaf wetness sensors have the disadvantage of not being painted by the manufacturer and requiring an in-situ calibration that cannot be easily performed by growers. Painting provides more precise wetness estimations by responding better to the onset and offset of wetness, and thus, it is recommended when integrating LW sensors into a DAS (Gillespie and Kidd 1978; Sentelhas et al. 2004b; Sentelhas et al. 2008). Calibration of these sensors can be performed in the field or the laboratory. Briefly, laboratory calibration is done by applying droplets of water to the sensor and registering the electric resistance values provided by the sensor (Sentelhas et al. 2004a). Field calibration requires on-site observations of dew onset and offset so a resistance threshold that distinguishes wet and dry is established (Rao et al. 1998). The establishment of resistance thresholds is essential for the use of Campbell 237-L

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sensors since we have observed a significant variation in the values below which a sensor considers a resistance observation as wet (Montone 2013). Given the advantages and disadvantages of each sensor and the importance of LW for DAS, the objective of this study was to compare the LW estimations of the recently improved Decagon dielectric sensors to those of Campbell 237-L sensors over three years in Florida.

Materials and Methods

Weather stations and data acquisition. All the sensors were located in the same weather station installed in Plant City, Fla. This weather station is maintained by FAWN, and data were retrieved from its database. The weather station was equipped with four LW sensors, two Decagon dielectric, and two Campbell 237-L sensors. The sensors were placed approximately 10 cm from each other at 30 cm over turfgrass and installed at a 45º angle. The two sets of Campbell and Decagon sensors were positioned adjacent to each other and installed on a PVC pipe bar centered at the base of the automated weather station. One set of sensors was placed equidistantly to the left and the other one to the right of the weather station. Leaf wetness (LW) data were acquired every 15 min. from 26 July 2017 to 29 Jan. 2020. A total of 88,096 observations for each of the four sensors was recorded for the analysis.

The comparison between sensors was performed based on a methodology adapted from Montone et al. (2016) and Kim et al. (2004). Comparisons were performed in pairs for every possible combination: Campbell sensor 1 vs. Campbell sensor 2; Decagon sensor 1 vs. Decagon sensor 2; Decagon sensor 1 vs. Campbell sensor 1; Decagon sensor 2 vs. Campbell sensor 1; Decagon sensor 1 vs. Campbell sensor 2; and Decagon sensor 2 vs. Campbell sensor 2.

Linear regression analysis, determination of Pearson's correlation coefficients, mean square error (MSE), and mean absolute error (MAE). Pairwise linear regression analyses were performed for every combination of sensors. When comparing different sensors, data from Campbell 237-L sensors were considered the standard and plotted on the x-axis of the regression. Maximum daily LW duration data for each sensor were considered for this analysis, which totaled 931 data points (days) for each sensor. The slope, intercept, coefficient of determination (R^2), and statistical significance level (*P*-value) were calculated for each combination of sensors. Pearson's correlation coefficients were also calculated for data obtained for each of the combinations of sensors described previously. The significance level for the correlation was also calculated. Daily LW duration values were also used to calculate the mean square error (MSE) and mean absolute error (MAE) of the relationship as done previously in a similar study (Montone et al. 2016). The errors were calculated in the unit of hours and hours^2 for MAE and MSE, respectively.

Contingency table and k agreement indices calculations. Every observation acquired at 15-min intervals was used for this step of the comparison. The data obtained by the Campbell 237-L sensors were considered the standard for this analysis when they were compared to Decagon dielectric sensors, as Campbell 237-L sensors were calibrated based on visual observations in a site-specific manner and were known to provide good LW estimations from previous research (Montone et al. 2016). Six four-cell contingency tables were designed, one for every pairwise comparison of the combinations previously described. The tables had true positives for wetness (W), true negative for wetness (i.e., dry – D), false positive (i.e., false wet – FW), and false negative (i.e., false dry – FD). The number of W, D, FD, and FW events was calculated in the unit of hours, as done for MAE. After the tables were designed, a *k* agreement index (Dietterich 2000) was calculated for each comparison of sensors. The equations below (Eq. 1, Eq. 2, and Eq. 3) describe how the *k* agreement index and its components were calculated.

[Eq. 1]

$$\theta_1 = \frac{W + D}{W + D + FW + FD}$$

[Eq. 2]

$$\theta_2 = \frac{(W + FD) * (W + FW)}{(W + FD + FW + D)^2} + \frac{(FW + D) * (FD + D)}{(W + FD + FW + D)^2}$$

[Eq. 3]

$$k = \frac{\theta_1 - \theta_2}{1 - \theta_2}$$

In which:

θ_1 = fraction of correct estimations,
W = true positives for wetness,
D = true negative for wetness, i.e., dry events,
FW = positive (i.e., false wet—FW),
FD = false negative (i.e., false—FD),
θ_2 = estimate of the probability that the LW estimations agree by chance, based on the contingency table counts,
k = *k* agreement index.

Results

The agreement between sensors of the same manufacturer (Campbell 1 vs. Campbell 2 and Decagon 1 vs. Decagon 2) was very good (Fig 1A and B, Table 1). The coefficient of determination (R^2) was 0.96 and 0.83 for the comparisons between Campbell and Decagon sensors, respectively (Fig 1-A, 1-B, Table 1). When comparing Decagon and Campbell sensors, however, the agreement was not as good (Fig. 1, Table 1). The R^2 of the relationships between Campbell and Decagon sensors ranged from 0.60–0.64 (Fig. 1C–F, Table 1). When comparing the regression line between the data points obtained from Campbell and Decagon sensors, an overestimation of LW duration by Decagon sensors in relation to the Campbell ones was revealed, especially at maximum daily LW duration lower than 20h (Fig. 1C–F). At approximately 30 h of LW duration, a slight shift towards underestimation was observed (Fig. 1C–F). Pearson's correlation coefficients for the sensor comparisons from the same manufacturer were high, both above 0.9 (Table 1). When comparing sensors from different manufacturers, the correlation coefficients were all approximately 0.8 (Table 1). Similarly, MAE estimations were low when comparing sensors from the same manufacturer, all below 1 h (Table 1). However, MAE increased to about 2.2 h when compared the different sensors (Table 1). The same pattern was observed for *k* agreement indices. Comparisons between Campbell sensors and Decagon sensors had very high *k* agreement indices of 0.96 and 0.92,

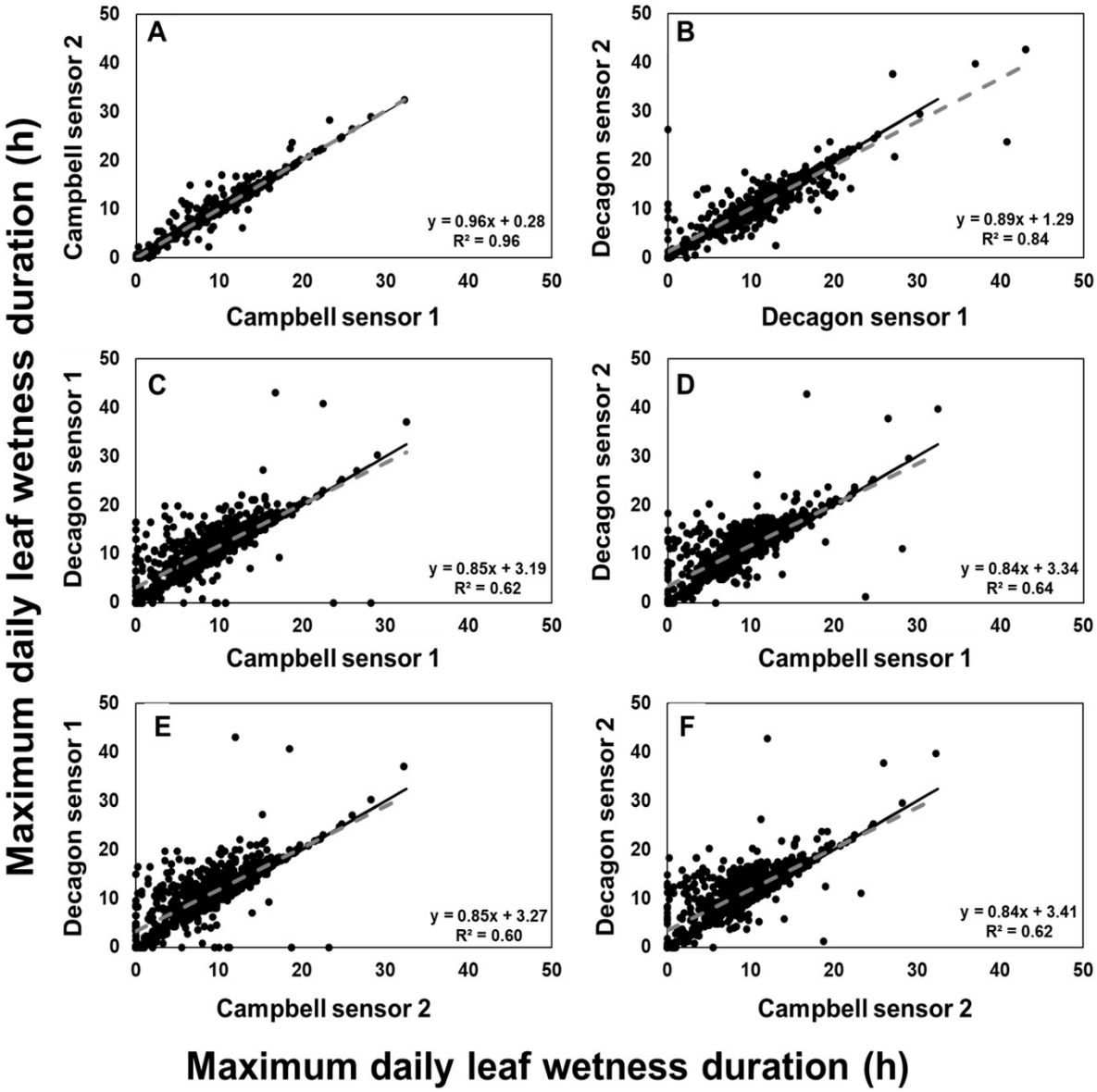

Fig. 1. Linear regression (gray dashed line) analysis between maximum daily leaf wetness duration (in hours) estimated by Campbell 237-L sensor 1 and Campbell 237-L sensor 2 (**A**), Decagon dielectric sensor 1 and Decagon dielectric sensor 2 (**B**), Campbell 237-L sensor 1 and Decagon dielectric sensor 1 (**C**), Campbell 237-L sensor 2 and Decagon dielectric sensor 1 (**D**), Campbell 237-L sensor 1 and Decagon dielectric sensor 2 (**E**), and Campbell 237-L sensor 2 and Decagon dielectric sensor 2 (**F**). The continuous black line represents the perfect agreement line (i.e., a R^2 of 1.0).

Table 1. Pearson's correlation coefficient (Pc), coefficient of determination of the linear regression (R^2), intercept and slope of the linear regression, and their respective estimation significance levels (*P* value) related to the comparison between Campbell 237-L and Decagon dielectric leaf wetness sensors. Mean square error (MSE), mean absolute error (MAE), and *k* agreement index of leaf wetness data acquired at 15-minute intervals by different sensors, specified in the comparison column.

	Correlation		Linear regression							*k* agreement
Comparison	Pc	*P* value	R^2	Intercept	*P* value	Slope	*P* value	MSE[z]	MAE[z]	index[y]
Campbell 1 vs. Campbell 2	0.98	< 0.0001	0.96	0.28	< 0.0001	0.96	< 0.0001	0.91	0.42	0.96
Decagon 1 vs. Decagon 2	0.92	< 0.0001	0.84	1.29	< 0.0001	0.89	< 0.0001	4.19	0.96	0.92
Campbell 1 vs. Decagon 1	0.80	< 0.0001	0.62	3.19	< 0.0001	0.85	< 0.0001	13.46	2.20	0.81
Campbell 1 vs. Decagon 2	0.80	< 0.0001	0.64	3.34	< 0.0001	0.84	< 0.0001	12.53	2.12	0.80
Campbell 2 vs. Decagon 1	0.77	< 0.0001	0.60	3.27	< 0.0001	0.85	< 0.0001	14.54	2.35	0.80
Campbell 2 vs. Decagon 2	0.79	< 0.0001	0.62	3.41	< 0.0001	0.84	< 0.0001	13.52	2.26	0.80

[z]Calculated based on the difference between daily maximum wetness duration estimated by each combination of sensors

[y]Calculated according to the methodology developed by Dietterich (2000).

respectively (Table 1). The *k* agreement indices of comparisons made between Campbell and Decagon sensors were all approximately 0.8 (Table 1). Our results do not indicate any loss in precision for the two types of sensors over the course of the three years of data analyzed in this study.

Discussion

While the Campbell 237-L sensors are the gold standard, the Decagon sensors can be viable options for weather stations used for plant disease risk calculation based on our results. These sensors provide LWD estimation within the acceptable MAE margin for application in DAS of approximately 2 h (Sentelhas et al. 2008) compared to in-situ calibrated Campbell 237-L sensors. The convenience of being painted and calibrated with a pre-established LW threshold makes the Decagon dielectric sensors suitable options to be installed in FAWN or grower-owned weather stations. The comparisons of the four sensors yielded very similar regression equations and results. The analysis of three-years of data was important to verify the durability of the sensors, i.e., to confirm if the measurements would lose accuracy over time. Growers and researchers should keep monitoring the data from weather stations to identify erroneous data in DAS, especially during critical periods for plant disease management in Florida.

In Florida, the Agroclimate decision-support system contains three DAS, intended for strawberry, citrus, and blueberry growers (Fraisse et al. 2016; Gama et al. 2021; Pavan et al. 2011; Perondi et al. 2020). They all rely on LW data to calculate and provide daily disease risk assessments to growers. LW sensors must be reliable when feeding information to the DAS once the implementation of disease management practices must be deployed as promptly as possible to maximize efficacy. It is also desirable for sensors to be durable to provide results for more than one season. Our results could be used by growers, extension specialists, and stakeholders when deciding which LW sensor to install in weather stations used for DAS application in Florida.

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—Scientific Note—

Flushing Pattern, Asian Citrus Psyllid Colonization and *Candidatus* Liberibacter asiaticus Transmission In Different Graft Combinations of Citrus

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Additional index words. huanglongbing, citrus greening, rootstock, scion, trifoliate orange

Citrus rootstocks are critical to a viable citrus industry because they influence tree health, vigor, yield, and fruit quality and therefore profitability of a citrus planting. In addition to their inherent horticultural characteristics and influence on the grafted tree, rootstocks may influence the colonization of the grafted scion by the Asian citrus psyllid (ACP), the vector of *Candidatus* Liberibacter asiaticus (*C*Las). *C*Las is a phloem-limited bacterium and associated with Huanglongbing (HLB), a disease that is devastating citrus industries worldwide. While most scion varieties are susceptible to HLB, some rootstocks are HLB-tolerant and allow the tree to cope better with the disease. This study investigates if psyllid colonization and *C*Las spread in a grafted citrus tree are associated with flushing patterns, psyllid preference, and the interaction between rootstock and scion.

Seven commercially important rootstock cultivars with different genetic backgrounds were self-grafted or grafted with 'Valencia' sweet orange (*Citrus sinensis*) and planted at the Southwest Florida Research and Education Center research farm in Immokalee, FL, where ACP and *C*Las were abundant. The rootstock cultivars included 'US-802', 'US-897', 'US-942', 'Carrizo', and 'Swingle', which are hybrids of citrus and trifoliate orange (*Poncirus trifoliata*), and sour orange (*C. aurantium*) and 'Cleopatra' mandarin (*C. reticulata*). Previous studies have documented that trifoliate orange and some of its hybrids are HLB-tolerant.

The experimental design was a split-plot with rootstock as the main plot and grafted scion as the subplot and 10 single-tree replications. Trees were planted in Mar. 2019. Trees were lightly pruned after planting and did not receive any insecticides during the trial period. The proportion of new flush and infestation with ACP were determined weekly after planting and during the flushing period from Feb–Mar. 2020 by counting the number of shoots with flush, the number of adult psyllids, and the presence of eggs and nymphs on 10 randomized shoots per tree. Fully matured leaves and fibrous roots were collected every 3 months after planting, DNA was extracted, and *C*Las titer levels were assessed by real-time polymerase chain reaction and expressed as Ct (cycle-threshold) values.

Flushing patterns varied significantly among scion cultivars with 'Valencia', sour orange, and 'Cleopatra' scions flushing earlier in the season than the other scions. Trees with 'Valencia' in the scion position were more attractive to the psyllids (Fig. 1) and

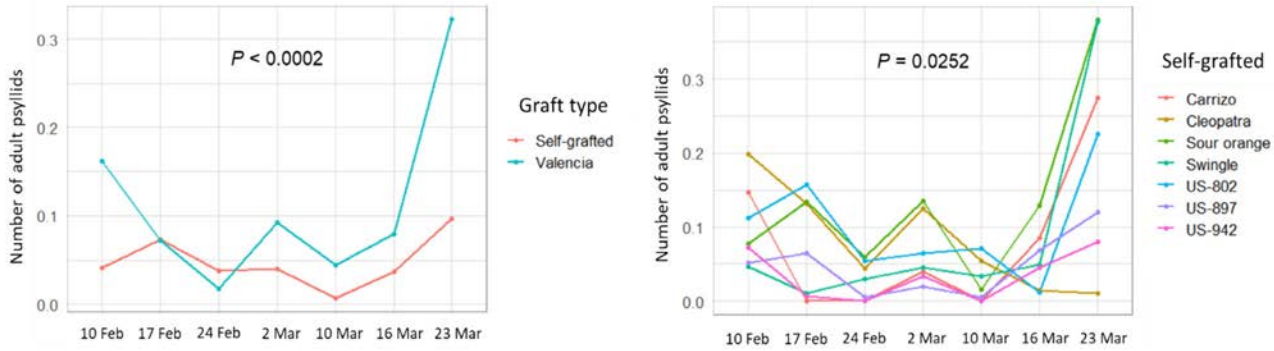

Fig. 1. Number of psyllids on different scion/rootstock combinations during February to March 2020. (Left): comparison of trees on rootstocks grafted with themselves (self-grafted) and grafted with 'Valencia'. (Right): comparison of self-grafted trees.

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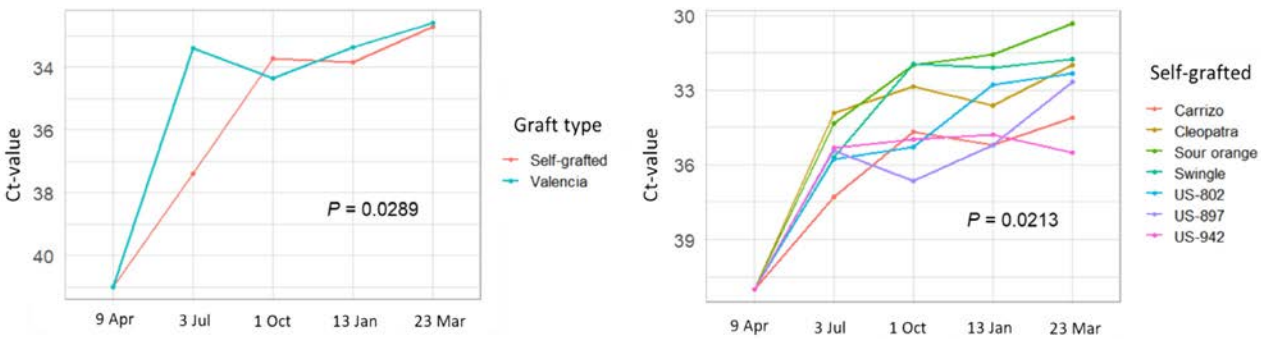

Fig. 2. Ct values of roots from trees composed of different scion/rootstock combinations during April 2019 and March 2020. (Left): comparison of trees on rootstocks grafted with themselves (self-grafted) and grafted with 'Valencia'. (Right): comparison of self-grafted trees.

had significantly higher incidences of eggs and nymphs, higher leaf *C*Las titers, and displayed more foliar disease symptoms than self-grafted trees. The rootstock cultivar did not have any significant effect on the grafted 'Valencia' scion for these variables. Leaf *C*Las titers were higher than root *C*Las titers regardless of the rootstock cultivar with no significant rootstock effect on the scion. In contrast, *C*Las titer levels of roots varied significantly among self-grafted trees (Fig. 2).

The flushing pattern was influenced by the cultivar in the scion position but not by the cultivar in the rootstock position. Psyllids were more attracted to 'Valencia' leaves than to leaves of the other cultivars, which may have contributed to the higher *C*Las titers measured in 'Valencia' leaves compared to the other cultivars. *C*Las titers were also influenced by the rootstock cultivar, but only in the roots of self-grafted trees. Taken together, the results suggest that psyllid colonization and leaf *C*Las titer levels of grafted trees are influenced predominantly by the scion cultivar, but that *C*Las levels of roots depend on the rootstock cultivar, which may affect the grafted tree tolerance to HLB in the long-term.

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—Scientific Note—

Citrus Nutrition Trends In the Florida Citrus Industry

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Additional index words. fertilizer, nutrient management

Since the discovery of Huanglongbing (HLB; citrus greening) in Florida, citrus tree health has been on a continual decline. There has been controversy if changes in nutrition management practices would be beneficial for HLB-affected trees. In the last few years, studies have shown that enhanced nutrition such as maintaining nutrient levels through small, frequent applications, customizing nutrition management for individual groves, and applying higher rates of micronutrients than previously recommended for healthy trees has been beneficial for HLB-affected trees (Vashisth and Livingston, 2019; Zambon et al., 2019; Vashisth, 2020).

In Oct. 2019, the citrus nutrition box program was initiated for Florida citrus growers. The primary objectives were to determine the current status and trends of nutrition levels and soil pH in commercial citrus groves and to demonstrate role of regular leaf nutrient analysis in fertilization management for HLB-affected groves. The program was designed to be a demonstration, not a scientific experiment.

For the first quarter, there were 75 samples collected and analyzed from 17 counties between Oct. 2019 and Jan. 2020. There was a wide range of variables such as variety and rootstock combinations, location, tree age, and tree health. Using the University of Florida Institute of Food and Agricultural Science (UF IFAS) recommended optimum nutrition ranges for healthy orange trees, nutrient leaf concentration averages were calculated on the statewide, regional, and county levels.

From a statewide perspective, all nutrients were in the optimum range except copper. Copper levels were high in several counties. The counties with the highest levels of copper are known for growing fresh fruit or are on the coastal regions versus counties with optimum ranges that are often known for growing processed fruit. This is thought to be caused by the last copper application for preventing citrus canker before harvest. The highest copper level was 97 on flame grapefruit.

All counties were in the optimum range for potassium, calcium, manganese, and zinc. St. Lucie County was slightly low in magnesium, but all other counties were in the optimum range for magnesium.

Iron levels were low in counties from the western and southern regions. The cause of the low iron was uncertain, but early considerations are soil type due to which iron was unavailable to the plant for uptake (Zekri, et al., 2018), possible nutrient competition during uptake (Morgan, et al., 2020), and use of lowly available iron fertilizer (such as non-chelated forms) (Basiouny et al., 1971). There were no high iron levels in any individual samples analyzed throughout the state.

High boron was observed in Indian River and Charlotte Counties. These two counties are on the east and west coasts respectively; therefore, the high boron levels were not likely regional. It is thought that growers may be trying additional boron applications in their groves based on previous research (Grosser and Barthe, 2015). Other considerations for high boron levels may be the possible buildup of boron over the years or little rainfall since samples were collected during the dry season. Of the 75 individual samples, 28% had high boron and no toxicity symptoms had been reported. The highest boron level was 206 ppm on 'Hamlin' oranges (*Citrus sinensis*).

Several counties were in the high range for nitrogen and phosphorus. Polk and Highlands Counties were the only two counties that were in the optimum range for all nutrients.

The optimum soil pH range for citrus is 6.0–6.5 (Morgan, et al, 2020), but the pH range for the samples analyzed varied (4.4–7.8 pH). An overall average on the county level indicated optimum or low levels. The lowest pH averages were observed in DeSoto and Charlotte Counties. Poor water quality caused by bicarbonates can be corrected by using acidification practices to reduce high pH levels (Graham, et al., 2015). Due to the alkaline soil in those areas, it is common for soil acidification practices to be used to lower soil pH.

Moving forward, the additional quarterly data collected from the nutrition box program samples will be evaluated to record seasonal and geographical leaf nutrient trends as well as regular leaf nutrient analysis in effective fertilization. The program will be repeated in 2020–21 to allow for a second year of observation. A three-year research field trial will begin in 2021 to determine trends and relationships of leaf concentrations.

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—Scientific Note—

PAPS: A Portable and Adjustable Prototype Sprayer to Analyze Fungal Biopesticide Deposition In the Greenhouse

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When evaluating spray leaf coverage of fungal-based biopesticides for insects associated with Huanglongbing management in citrus, a significant amount of data is needed to: 1) analyze the efficiency of the sprayer in applying a uniform amount of biopesticide, and 2) evaluate the biopesticide efficacy for management of pest populations. To collect this data, it is necessary to have controlled environmental and field conditions. These parameters which include the absence of wind, availability of the spraying machine, field management strategies, etc. significantly increase the cost and time required to obtain meaningful research outcomes. In addition, cost-efficient sprayer devices that can simulate the spraying patterns of field machines in a lab setting are not available. The goal of this study was to develop a cost-efficient portable and adjustable prototype sprayer (PAPS), that can be used under controlled environmental conditions, including laboratory and greenhouse, for the evaluation of biopesticide leaf coverage.

To define the spraying simulation patterns in PAPS, two commonly used sprayer machines for citrus management applications were selected the air-blast (AB) sprayer and ultra-low volume (ULV) sprayer. AB is characterized in distributing the pesticide by creating a wind blast hitting the leaves under a specified pressure and, ULV by producing a mist of very fine droplets of pesticide directed through a set of ventilators.

To assess the feasibility of PAPS, we calibrated multiple variables including droplet size, air speed, and pesticide incidence angle. The initial calibration of the AB and ULV spray application machines was performed at the Florida Research Center for Agricultural Sustainability (FLARES) in Vero Beach, FL. The conditions used by crop managers in a commercial citrus field were reproduced. Calibration of PAPS was performed at the University of Florida, Indian River Research and Education Center (IRREC), in Fort Pierce, FL, under greenhouse conditions. Sensitive paper and a handheld anemometer were used for the collection of droplet size-distribution and air speed data, respectively. Measurements were collected at a wall with sensitive paper located 2.1 m from the prototype application device. The sensitive paper was placed following a quadrant across the PAPS nozzles wet pattern. Preliminary results using water showed that, based on droplet size estimations, this prototype can represent the conditions of the AB and ULV sprayer in the laboratory or greenhouse. A summary of the field and the prototype sprayer calibrations are presented in Table 1.

The observed spray droplet sizes were within the range of 131.6 to 390 μm and 31.6–139.3 μm for PAPS-AB and PAPS-ULV, respectively. This confirms that PAPS can accurately represent the conditions of the AB and ULV field application machines. It is important to understand that if the distance from PAPS to the target is modified, the pressure and droplet size would be modified as well. Results of air speed tests indicated that the average difference in speed between AB and ULV for both the field application machines and PAPS is nearly 30%. Values in PAPS are lower due to the reduction in scale from the field application machines. The implementation of PAPS in the greenhouse is a cost-effective and time saving solution to gather sprayer efficiency data, determine leaf coverage, and indirectly evaluate the effects of biopesticides for management of pest populations. A 360 visualization of PAPS can be found at: <https://www.youtube.com/watch?v=T0jMVDacXek>. Future work includes the evaluation of the relationship between changes in incidence angle and leaf coverage, and laboratory assessment of pest control comparing the field machines with PAPS.

Table 1. Sprayer calibration results of air blast (AB) and ultra-low volume (ULV) sprayers in the field vs. portable and adjustable prototype sprayer (PAPS) calibration results (PAPS–AB and PAPS–ULV) using water.

Parameter	AB	ULV	PAPS–AB	PAPS–ULV
Droplet size (μm)	131.6–390	31.6–139.3	200	110
Air speed (m/s)	53.65	41.12	4–15	6.71

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Field Performance of 'Hamlin' Orange Trees Grown on Various Rootstocks in Huanglongbing-endemic Conditions

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***Abstract*. Most of the commercially important citrus scion cultivars are susceptible to Huanglongbing (HLB), which is the most devastating disease the citrus industry has ever faced. Because the rootstock can influence the performance of the scion in various ways, including disease and pest tolerance, use of superior rootstocks can assist citrus growers with minimizing the negative effects of HLB. The objective of this study was to assess rootstock effects on the horticultural performance and early production potential of 'Hamlin' sweet orange (*Citrus sinensis*) trees in commercial field settings under HLB-endemic conditions. Two field trials were conducted in different locations in Central and Southeast Florida. The trials were established in 2015 and included 32 diverse diploid and tetraploid rootstock cultivars and advanced selections. One trial was performed in Highlands County, FL, on a poorly drained flatwoods-type site. Another trial was performed in Polk County, FL, on a well-drained sandy Central Florida Ridge site. Horticultural traits including tree height, canopy volume, trunk diameter, canopy health, leaf nutrient content, yield, and fruit quality were assessed during the 2018–19 and 2019–20 production years. Significant differences were found among trees on different rootstocks for most of the measured traits, particularly tree vigor and productivity, but rootstock effects also varied by location. Rootstocks that induced large tree sizes, such as the diploid mandarin × trifoliate orange hybrids 'X-639', 'C-54', 'C-57', and 'C-146', also induced higher yield, but with lower yield efficiency. Most of the tetraploid rootstocks significantly reduced tree size, among which 'Changsha+Benton', 'Green-3', 'Amb+Czo', 'UFR-3', and 'UFR-5' induced high yield efficiency. Therefore, these rootstocks have the potential to be used in high-density plantings. However, trees on some of these small size-inducing rootstocks had a higher mortality rate and were more vulnerable to tropical force winds. This study provides important information for the selection of rootstocks with the greatest production potential in an HLB-endemic environment, especially during the early years of production.**

The success of modern citrus industries relies on the suitable combination of superior scions and rootstocks and their adaptability to diverse soil and environmental conditions (Castle, 2010; Castle and Gmitter, 1998). The importance of rootstocks in the citrus industry has increased since they were recognized to provide tolerance against various pests and diseases. Use of sour orange rootstock to protect previously own-rooted citrus trees from *Phytophthora* disease and the use of trifoliate orange-type rootstocks to protect trees from citrus tristeza virus (CTV) are the major events (Roistacher et al., 2010) that shaped the citrus industry in Florida and other production areas. Furthermore, rootstocks can generate tolerance against various abiotic stresses such as drought, flooding, extreme pH conditions, extreme temperatures, and salinity (Cimen and Yesiloglu, 2016). The influences of rootstocks on the precocity of flowering and fruiting and on other horticultural traits of the scion, such as tree vigor, nutrient uptake, yield, and fruit quality, have also been established (Castle, 2010; Forner-Giner et al., 2003; Nimbolkar et al., 2016; Webster, 1995; Wutscher, 1979). Therefore, rootstock selection is crucial for determining the sustainability, productivity, and long-term profitability of a citrus orchard (Castle, 2010; Nimbolkar et al., 2016; Webster, 1995).

The Florida citrus industry grew from the introduction of seeds and plants of sour orange, sweet orange, lemon, lime, and citron by the Spanish in the 16th century, and the use of grafted trees commenced in the1830s (Castle et al., 1993). Since then, the industry has changed over time to adapt to different pests and diseases and environmental events. The introduction of the destructive disease HLB is the most recent event, and it continues to impact citrus production not only in Florida but also worldwide.

HLB is a disease associated with the phloem-limited bacterium *Candidatus* Liberibacter asiaticus that is transmitted by Asian citrus psyllids (*Diphorina citri*); it was found in Florida in 2005 (Gottwald et al., 2007). Since the confirmation of HLB in Florida, the HLB incidence has rapidly increased from 0.2% in 2006 to essentially 100% at present (Graham et al., 2020). This has resulted in tremendous economic losses of more than 70% in citrus production (year 2018–19) compared with the era before HLB (year 2003–04) (FDACS, 2020a). Different disease management strategies include vector exclusion (Ferrarezi et al., 2019; Schumann and Singerman 2016), vector control (Gottwald et al., 2007; Stansly et al., 2014), nutrient management (Rouse et al., 2017; Stansly et al., 2014; Zambon et al., 2019), and the application of plant defense inducers and antibiotics (Hu and Wang 2016; Hu et al., 2018). However, there are difficulties adopting some of these technologies under large-scale field conditions because of environmental or economic constraints.

Most citrus scion cultivars are HLB-susceptible and decline quickly if not managed properly (Miles et al., 2017). In contrast, several rootstock cultivars are tolerant to HLB and can render grafted trees more productive in the presence of the disease (Albrecht and Bowman, 2012; Bowman and McCollum, 2015; Bowman et al., 2016a, 2016b). The recognition of the importance of rootstocks for sustaining citrus production in an HLB-endemic environment has led to a shift in rootstock use in Florida, such as the preference for US-942 and other rootstocks over Swingle, which for decades has been the most propagated rootstock in Florida (FDACS, 2020b). The demand for other rootstocks bred to combine many of the most desired rootstock traits is increasing and necessitates field evaluation in a commercial production environment.

Bowman and Joubert (2020) identified 21 rootstocks as major world rootstocks; of

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which, 10 are naturally occurring species or hybrids and the rest are hybrids from breeding programs with one of the parents being *Poncirus trifoliata*. Therefore, there are opportunities for developing more sophisticated hybrid rootstocks by using modern breeding tools that allow the incorporation of superior traits from diverse parents (Germanà et al., 2020). Modern technologies such as molecular markers and somatic hybridization have emerged and have been successfully implemented, thus waiving many barriers associated with conventional breeding methods (Grosser et al., 2010; Khan and Kender, 2007).

We compared the field performance of several advanced experimental rootstock selections with other commercially used rootstocks. Many of the rootstocks used are tetraploid hybrids developed from either somatic hybridization of two complementary parents or crosses at the tetraploid level using somatic hybrid parents. The other rootstocks are diploid hybrid cultivars, except for sour orange (*C. aurantium*). The rootstocks included in our study have a wide range of genetic diversity, including combinations of trifoliate orange, mandarin, lime, lemon, pummelo, grapefruit, citrange, and sour orange. The objective of this study was to evaluate the effect of rootstocks on the horticultural performance of 'Hamlin' orange and to assess their potential for commercial production in an HLB-endemic environment.

Materials and Methods

Plant material

The rootstocks were developed in Florida (University of Florida), California (University of California, Riverside), and Spain (Valencian Institute of Agricultural Research). They contain sexual and somatic hybrids from a wide range of different germplasms (Table 1). The same rootstocks were used in both trials, except for sour orange, which was only included in one trial. Rootstock liners were grown from seeds and grafted with 'Hamlin' orange (*C. sinensis*) scion using standard procedures (Albrecht et al., 2017; Lewis and Alexander, 2008). Trees were produced in a commercial citrus nursery (Lykes Citrus, Basinger, FL) and planted in 2015 in commercial citrus orchards at the locations described.

Trial locations

Two field trials were conducted, with each in a separate location with different environmental conditions but under the same commercial management (Lykes Bros. Inc.). The first trial was established in Apr. 2015 near Fort Basinger (lat. 27.373321°N, long. –81.135209°W) in Highlands County, FL. The second trial was established in June 2015, near Lake Wales (lat. 27.935447°N, long. –81.49927°W) in Polk County, FL. Because HLB has been endemic in Florida since 2013 (Graham et al., 2020), the HLB incidence in both production areas was 100% at the time of planting.

The soil type at the Fort Basinger location is a poorly drained sandy Entisol with Spodosol-like properties, whereas that at the Lake Wales location is a well-drained sandy Entisol (Obreza and Collins, 2008). Both soil types have little organic matter and a low cation exchange capacity. Random soil samples were collected across each trial site to a depth of 25 cm near the drip line of trees and pooled for physicochemical analysis (Waters Agricultural Laboratories, Inc., Camilla, GA). An analysis showed an organic matter content of 0.72%, pH of 5.7, and cation exchange capacity (CEC) of 4.5 meq/100 g for the Basinger location, and an organic matter content of 0.34%, pH of 5.5, and a CEC of 3.2 meq/100 g for the Lake Wales location. Sand, silt, and clay contents were 95.2%, 0.8%, and 4%, respectively at Basinger, and 99.2%, 0.8%, and 0%, respectively, at Lake Wales. Thirty-two (trial 1) and 31 (trial 2) different rootstocks were used.

Experimental design

The experimental design was completely randomized, with six replications arranged in linear plots of eight trees each. Trees at the Basinger location were planted in double-row raised beds separated by furrows at a spacing of 8 ft (2.4 m) along the rows and 25 ft (7.6 m) between the rows. Trees at the Lake Wales location were planted in nonbedded single rows at a spacing of 8 ft (2.4 m) along the rows and 22 ft (6.7 m) between the rows.

Table 1. Rootstock names and their parentage.

Rootstock	Parentage
Tetraploids	
6058 + 2071-02-2*	*Citrus aurantium*+*C. limonia* 'Rangpur' × *C. reticulata* 'Cleopatra'+*Poncirus trifoliata*
Amb+Benton*	*C. amblycarpa*+*Citroncirus* spp. 'Benton'
Amb+Czo*	*C. amblycarpa*+*Citroncirus* spp. 'Carrizo'
Changsha+Benton*	*C. reticulata* 'Changsha'+*Citroncirus* spp. 'Benton'
Green-3*	*C. reticulata* 'Nova'+*C. maxima* 'Hirado Buntan' × *C. aurantium*+*Citroncirus* spp. 'Carrizo'
Green-7*	*C. reticulata* 'Nova'+*C. maxima* 'Hirado Buntan' × *C. aurantium*+*Citroncirus* spp. 'Carrizo'
Orange-14*	*C. reticulata* 'Nova'+*C. maxima* 'Hirado Buntan' × *C. reticulata* 'Cleopatra'+*P. trifoliata*
White-1*	*C. reticulata* 'Nova'+*C. maxima* 'Hirado Buntan' × *C. sinensis* 'Succari'+*P. trifoliata*
Sorp+Sh-991*	*C. aurantium*+*C. limonia* 'Rangpur' × *C. reticulata* 'Cleopatra'+*P. trifoliata*
Wgft+50-7*	*C. paradisi* 'White'+*P. trifoliata* '50–7'
UFR-1	*C. reticulata* 'Nova'+*C. maxima* 'Hirado Buntan' × *C. reticulata* 'Cleopatra'+*P. trifoliata*
UFR-2	*C. reticulata* 'Nova'+*C. maxima* 'Hirado Buntan' × *C. reticulata* 'Cleopatra'+*P. trifoliata*
UFR-3	*C. reticulata* 'Nova'+*C. maxima* 'Hirado Buntan' × *C. reticulata* 'Cleopatra'+*P. trifoliata*
UFR-4	*C. reticulata* 'Nova'+*C. maxima* 'Hirado Buntan' × *C. reticulata* 'Cleopatra'+*P. trifoliata*
UFR-5	*C. reticulata* 'Nova'+*C. maxima* 'Hirado Buntan' × *C. sinensis* 'Succari'+*P. trifoliata*
UFR-6	*C. reticulata* 'Changsha'+*P. trifoliata* '50-7'
UFR-17	*C. reticulata* 'Nova'+*C. maxima* 'Hirado Buntan' × *C. aurantium*+*Citroncirus* spp. 'Carrizo'
Diploids	
C-146	*C. reticulata* 'Sunki' × *P. trifoliata* 'Swingle'
C-22 ('Bitters')	*C. reticulata* 'Sunki' × *P. trifoliata* 'Swingle'
C-54 ('Carpenter')	*C. reticulata* 'Sunki' × *P. trifoliata* 'Swingle'
C-57 ('Furr')	*C. reticulata* 'Sunki' × *P. trifoliata* 'Swingle'
ES-1	*C. reticulata* 'Cleopatra' × *P. trifoliata*
ES-2	*C. reticulata* 'Cleopatra' × *P. trifoliata*
ES-3	*C. reticulata* 'Cleopatra' × *P. trifoliata*
ES-5	*C. reticulata* 'King' × *P. trifoliata*
ES-4	*C. reticulata* 'King' × *P. trifoliata*
ES-6	*C. reticulata* 'King' × *P. trifoliata*
US-897	*C. reticulata* 'Cleopatra' × *P. trifoliata* 'Flying dragon'
ES-7	*C. volkameriana* × *P. trifoliata*
Sour orange	*C. aurantium*
Swingle	*C. paradisi* 'Duncan' × *P. trifoliata*
X-639	*C. reticulata* 'Cleopatra' × *P. trifoliata* 'Rubidoux'

+ indicates somatic hybridization (allotetraploid). × indicates sexual hybridization (diploid or tetraploid).
*Experimental rootstocks.

Irrigation was automated by under-tree microjets. A controlled-release fertilizer (17N–6P–12K; Harell's LLC, Lakeland, FL) was applied at rates of 1 lb (0.45 kg), 2 lb (0.91 kg), and 3 lb (1.36 kg) per tree in years 1, 2, and 3 after planting, respectively. Starting in year 4, dry fertilizer (12N–0P–13K; Howard Fertilizer, Lake Placid, FL) was applied in the fall, spring, and summer of every year at a rate of 36 lb N per acre (40 kg/ha) per application. Liquid fertilizer (7N–2P–8K; Howard Fertilizer) was applied annually at a rate of 60 lb N per acre (67 kg/ha) during January to June and September to October. Weed management and insecticide applications were performed according to the grower's standards and were similar for both locations.

Plant assessments

Canopy health, tree survival, and leaning. Canopy color, canopy thickness, and foliar HLB disease symptoms were determined during Nov. 2019 by visual assessment of the third and sixth trees of each replicated plot. Canopy color and canopy thickness were rated on a scale of 1 to 5, with 1 representing the worst and 5 representing the best. Foliar disease symptoms (HLB disease index) were rated on a scale of 1 to 5, with 1 representing the best (no foliar disease symptoms) and 5 representing the worst (75% to 100% of the canopy showing foliar disease symptoms); ratings of 2, 3, and 4 represented 1% to 25%, 25% to 50%, and 50% to 75%, respectively, of the affected canopy. HLB symptoms included blotchy mottling of leaves, chlorosis, and other abnormalities associated with HLB (Bove, 2006). Eight ratings per tree were performed by dividing the tree into four quadrants on each side of the row, and the average was calculated for each tree. All assessments were conducted in Sept. 2019.

Dead and missing trees were counted in each plot of eight trees, and the tree survival was expressed as a percent. In 2017, hurricane Irma crossed Florida, which presented the opportunity to assess rootstock resistance to wind-induced leaning. The number of leaning trees in each plot was counted and expressed as a percent. A tree was defined as leaning when the angle between trunk and soil was less than 70°.

Tree size. Tree size measurements included tree height, canopy spread, and scion and rootstock trunk diameters. Measurements were conducted in Nov. 2019 using the third and sixth trees of each replicated tree plot. Tree height was measured using a digital measuring pole (Sokkia, Mississauga, ON, Canada) from the soil surface to the top of the tree excluding any erratic shoots. Canopy spread was measured parallel and perpendicular to the row using a measuring tape, and canopy volume (m^3) was calculated using the formula reported by Wutscher and Hill (1995): canopy volume = (diameter^2 × height)/4. Scion and rootstock trunk circumferences were measured at 5 cm above and below the graft union using a measuring tape. Trunk diameters (d) were determined using the circle circumference (C) formula $C = \pi \times d$.

Yield and fruit quality. Fruits were harvested from the third and the sixth trees in each replicated plot in 2018–19 and from all eight trees in 2019–20. Harvest dates were 22 Jan. 2019 and 21 Jan. 2020 (Lake Wales) and 18 Dec. 2018 and 8 Jan. 2020 (Basinger). Fruits were weighed using a digital scale (CW P-150; CAS, East Rutherford, NJ) and the average yield per tree was calculated. Yield efficiency was calculated by dividing the yield per tree (kg) by the canopy volume (m^3). Before harvest, a random fruit sample of 48 fruits per replicated plot was collected and analyzed at the Juice Processing Pilot Plant, Citrus Research and Education Center, University of Florida, Institute of Food and Agricultural Sciences, Lake Alfred, FL. The average fruit weight was determined using a digital scale. Juice was extracted using a pinpoint extractor (JBT, Chalfont, PA), and the fruit juice percentage was calculated. The total soluble solids (TSS) and acid content were determined using standard procedures.

Leaf nutrient analysis. Leaves were collected from the third and the sixth trees of each replicated plot in Sept. 2019. A random sample of 16 mature leaves per tree were collected from nonfruiting spring flush, resulting in 32 leaves per replicated plot. The leaf samples were sent to Waters Agricultural Laboratories (Camilla, GA) for macronutrient and micronutrient concentration analyses. Total nitrogen (N) concentration was determined using the Dumas combustion method modified by Sweeney (1989). For phosphorus (P), sulfur (S), potassium (K), zinc (Zn), magnesium (Mg), calcium (Ca), iron (Fe), manganese (Mn), copper (Cu), and boron (B), the leaves were digested using nitric acid and hydrogen peroxide solution and analyzed by an inductively coupled argon plasma (ICAP) analysis (Huang and Schulte, 1985).

Statistical analysis

A one-way analysis of variance was conducted for all variables measured, and the comparison of means was performed by Tukey's honestly significant difference test. Pearson correlation coefficients were calculated among selected response variables to test their associations. Differences were defined as statistically significant when $P < 0.05$. Data were analyzed using Rstudio version 1.3.1093 (R Core Team, 2020).

Results

Canopy health, tree survival, and leaning

The rootstock effect was significant for foliar disease symptoms and canopy thickness in both locations (Supplemental Table 1). In general, most trees looked healthy, with average disease indices not exceeding 1.6. At the Basinger location, trees on 'Amb+Benton' had a significant lower HLB disease index (1.2) than trees on 'ES-3' (1.6). At the Lake Wales location, trees on 'UFR-5' had a significant lower disease index (1.1) than trees on 'ES-7' (1.5). Most rootstocks induced thick canopies at either location. At the Basinger location, 'C-54' induced a significantly thicker canopy (4.8) than 'Changsha+Benton' (4.3). At the Lake Wales location, most rootstocks induced significantly thicker canopies (4.6–4.8) than 'Green-3' (3.8). Canopy color ratings ranged from 4.7 to 4.9, but there were no significant differences among rootstocks.

The rootstock significantly affected tree survival and leaning (Table 2). The survival rate was 98%, on average, in both locations, and significant rootstock effects were only found for the Basinger location, where trees on 'Green-3' had a significantly lower survival rate (85.4%) compared with most of the other rootstocks. At the Basinger location, the percentage of leaning trees was highest for trees on 'Amb+Benton' (41.7%), followed by 'C-22' (33.3%) and 'Changsha+Benton' (31.3%), and no leaning was observed for trees on 'Green-7', 'UFR-2', 'Wgft+50-7', and 'UFR-5'. At the Lake Wales location, trees on 'Amb+Benton', 'Changsha+Benton', and 'C-22' had the highest percentage of leaning (52.1% to 66.7%), whereas less than 10% of trees leaned on most other rootstocks.

Across both locations, the lowest percentage of survival was found for trees on 'Green-3' (90.6%). The percentage of leaning trees was highest for trees on 'Amb+Benton' (54.2%), followed by trees on 'Changsha+Benton' and 'C-22' (42.7%). The leaning of trees at the Basinger location and the Lake Wales location was significantly and inversely correlated with tree height ($R = -0.24$ and $R = -0.35$) and canopy volume ($R = -0.27$ and $R = -0.30$). A significant but weak correlation was also found between tree survival and canopy volume ($R = 0.15$ and $R = 0.24$).

Tree size

Tree height, canopy volume, scion trunk diameter, and scion-to-rootstock trunk diameter ratio (SRR) were significantly influenced by rootstock in both locations (Table 3). 'X-639', 'C-54', 'C-57', and 'C-146' induced the tallest trees (2.3 m) at the Basinger location and were among the rootstocks producing the tallest trees (2.2–2.3 m) at the Lake Wales location. 'Green-3', 'Changsha+Benton', and 'Amb+Benton' produced the smallest trees (1.5–1.6 m) at the Basinger location and were among the rootstocks producing the smallest trees (1.4–1.6 m) at the Lake Wales location.

Similar results were found for the canopy volume, which was largest for trees on 'X-639' and 'C-54' (2.8–3.1 m^3) at the Basinger location and for trees on 'X-639', 'C-54', and 'C-57' (3.0–3.3 m^3) at the Lake Wales location. Trees on 'UFR-3', 'Green-3', and 'Changsha+Benton' produced the smallest canopy volume (1.0–1.1 m^3) at the Basinger location, and together with 'Sorp+Sh-991' at the Lake Wales location (0.8–1.0 m^3).

Scion trunk diameters were largest in trees on 'C-54' and 'C-57' (8.5 cm) at the Basinger location and on 'C-57' (8.5 cm) at

Table 2. Tree survival and leaning of 'Hamlin' orange trees on different rootstocks.

	Trial 1 (Basinger)		Trial 2 (Lake Wales)		Avg	
Rootstock	Survival (%)	Leaning (%)	Survival (%)	Leaning (%)	Survival (%)	Leaning (%)
Amb+Benton	93.8 ab	41.7 a	100	66.7 a	96.9 a-c	54.2 a
Changsha+Benton	97.9 a	31.3 a-c	95.8	54.2 ab	96.9 a-c	42.7 ab
C-22	97.9 a	33.3 ab	100	52.1 a-c	99.0 ab	42.7 ab
6058+2071-02-2	100 a	20.8 a-d	100	29.2 b-d	100 a	25.0 bc
UFR-4	100 a	20.8 a-d	100	27.1 b-d	100 a	24.0 bc
UFR-6	97.9 a	18.8 a-d	100	27.1 b-d	99.0 ab	22.9 bc
Sorp+Sh-991	100 a	16.7 a-d	97.9	27.1 b-d	99.0 ab	21.9 bc
UFR-17	93.8 ab	12.5 a-d	97.9	25.0 b-d	95.8 a-c	18.8 bc
Green-3	85.4 b	16.7 a-d	95.8	16.7 cd	90.6 c	16.7 c
ES-7	97.9 a	10.4 b-d	100	16.7 cd	99.0 ab	13.5 c
UFR-3	91.7 ab	16.7 a-d	95.8	8.3 d	93.8 a-c	12.5 c
US-897	100 a	12.5 a-d	100	12.5 d	100 a	12.5 c
UFR-1	100 a	8.3 b-d	100	12.5 d	100 a	10.4 c
White-1	89.6 ab	6.3 b-d	95.8	14.6 d	92.7 bc	10.4 c
ES-4	100 a	8.3 b-d	100	12.5 d	100 a	10.4 c
Amb+Czo	100 a	8.3 b-d	100	10.4 d	100 a	9.4 c
ES-2	100 a	12.5 a-d	100	4.2 d	100 a	8.3 c
C-54	100 a	2.1 cd	100	12.5 d	100 a	7.3 c
C-57	100 a	10.4 b-d	100	4.2 d	100 a	7.3 c
Green-7	100 a	0 d	97.9	12.5 d	99.0 ab	6.3 c
ES-6	100 a	2.1 cd	100	7.5 d	100 a	4.6 c
UFR-5	97.9 a	0 d	100	8.3 d	99.0 ab	4.2 c
C-146	100 a	6.3 b-d	100	2.1 d	100 a	4.2 c
ES-3	97.9 a	2.1 cd	100	6.3 d	99.0 ab	4.2 c
ES-5	100 a	6.3 b-d	100	2.1 d	100 a	4.2 c
Orange-14	100 a	2.1 cd	100	4.2 d	100 a	3.1 c
UFR-2	97.9 a	0 d	95.8	4.2 d	96.9 a-c	2.1 c
Wgft+50-7	100 a	0 d	97.9	4.2 d	99.0 ab	2.1 c
ES-1	97.9 a	2.1 cd	100	2.1 d	99.0 ab	2.1 c
X-639	97.9 a	4.2 b-d	100	0 d	99.0 ab	2.1 c
Swingle	97.5 a	2.5 b-d	100	0 d	98.8 ab	1.3 c
Sour orange	97.9 a	2.1 cd	—	—	—	—
F value	3.14***	3.35***	1.497	6.03***	3.78***	8.76***

Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

the Lake Wales location. The smallest scion trunk diameters were found in trees on 'Green-3' (5.3 cm) at the Basinger location and in trees on 'UFR-3' (5.1 cm) at the Lake Wales location. The SRR was highest (0.86) for trees on rootstocks 'ES-5' and sour orange at the Basinger location and for trees on 'Changsha+Benton' (0.88) and 'Amb+Czo' (0.87) at the Lake Wales location. The lowest SRR (0.63) was found for the trees on 'Swingle' at both locations along with trees on 'Wgft+50-7' (0.63) at the Basinger location and 'UFR-3' (0.65) at the Lake Wales location.

Tree height was significantly correlated with both canopy volume and scion trunk diameter at the Basinger location (R = 0.92 and R = 0.88, respectively) and the Lake Wales location (R = 0.89 and R = 0.85, respectively).

Fruit yield

Fruit yield was measured in production years 2018–19 and 2019–20, and the cumulative yield was calculated. Rootstock effects on yield were significant for both years and at both locations (Table 4). At the Basinger location, the average yield was nearly threefold lower in 2018–19 (5.6 kg/tree) than in 2019–20 (15.4 kg/tree). Yield per tree ranged from 2.8 kg/tree for trees on 'ES-5' to 8.5 kg/tree for trees on 'US-897' in 2018–19, and from 9.6 kg/tree for trees on 'UFR-3' to 20.2 kg/tree for trees on 'ES-1' in year 2019–20; however, separation of means was not significant in either production year.

The average yield at the Lake Wales location was similar in both production years (Table 4). 'UFR-5' and 'ES-1' induced the highest yields per tree in 2018–19 (20.2 and 18.0 kg), and 'X-639' and 'C-54' induced the highest yields per tree (19.9 and 18.3 kg) in 2019–20. The lowest yields per tree were induced by 'Sorp+Sh-991', 'Wgft+50-7', 'UFR-3', and 'Amb+Benton' (4.6–6.8 kg) in year 2018–19, and by 'Changsha+Benton' and 'Sorp+Sh-991' (4.6 and 4.9 kg) in 2019–20.

Across both locations, the most productive rootstocks in terms of average cumulative yield were 'ES-1' (29.5 kg/tree), followed by 'UFR-5', 'C-54', and 'X-639' (27.8–28.0 kg/tree). The least productive rootstocks were 'UFR-3' (13.1 kg), followed by 'Sorp+Sh-991' (14.1 kg), 'Changsha+Benton' (15.3 kg), and 'Amb+Benton' (15.6 kg/tree). Fruit yield per tree was significantly correlated with canopy volume at the Basinger location (R = 0.39) and at the Lake Wales location (R = 0.66).

Yield efficiency

The rootstock effect was significant for yield efficiency in both locations and both production years (Table 5). In 2018–19, yield efficiency at the Basinger location ranged from 2.1 kg/m^3 for trees on 'X-639' to 9.9 kg/m^3 for trees on 'Amb+Benton', but the mean separation was not significant. In 2019–20, the average yield efficiency was highest for trees on 'Green-3' (14.3 kg/m^3) and lowest for trees on 'C-146', 'C-54', and 'C-22' (4.3–5.4 kg/m^3).

At the Lake Wales location, 'UFR-5', 'Green-3', and 'Changsha+Benton' induced the highest yield efficiency (15.7–17.3 kg/m^3) and 'ES-2', 'ES-3', 'C-57', and 'UFR-2' induced the lowest (6.1–6.2 kg/m^3) in 2018–19. In 2019–20, 'Green-3' induced the highest yield efficiency (10.5 kg/m^3) and 'UFR-6', 'UFR-17', 'ES-6', 'UFR-4', 'UFR-2', 'ES-3', and 'ES-2' induced the lowest yield efficiency (4.2–5.1 kg/m^3).

Across both locations and years, the highest average yield efficiency was induced by 'Green-3' (11.9 kg/m^3), followed by 'Amb+Benton' (10.5 kg/m^3), 'Changsha+Benton' (10.2 kg/m^3), and 'ES-4' (10.0 kg/m^3), whereas the lowest yield efficiency was induced by 'ES-2' (4.8 kg/m^3), followed by 'C-146', 'ES-3', and 'X-639' (5.2–5.4 kg/m^3). Yield efficiency was significantly and inversely correlated with canopy volume at the Basinger location (R = –0.59) and the Lake Wales location (R = –0.30).

Fruit quality

Fruit quality variables were measured in the production year 2019–20. The rootstock effect was significant for most variables in both locations. In the Basinger trial, 'Green-3' induced the highest weight fruit (174 g) and 'ES-2' induced the lowest fruit weight (140 g) (Table 6). The juice percentage was

Table 3. Tree size of 'Hamlin' trees on different rootstocks.

	Trial 1 (Basinger)				Trial 2 (Lake Wales)			
Rootstock	Ht (m)	Scion trunk diam (cm)	Canopy vol (m^3)	SRR	Ht (m)	Scion trunk diam (cm)	Canopy vol (m^3)	SRR
X-639	2.3 a	7.9 a-e	2.8 ab	0.77 b-h	2.3 ab	8.3 ab	3.3 a	0.75 g-l
C-54	2.3 a	8.5 a	3.1 a	0.79 a-g	2.3 a-c	8.1 a-c	3.0 a-c	0.81 a-h
C-57	2.3 a	8.5 a	2.6 a-d	0.80 a-e	2.3 a	8.5 a	3.1 ab	0.79 c-j
C-146	2.3 a	8.2 ab	2.8 a-c	0.75 c-h	2.2 a-d	7.9 a-d	2.6 a-e	0.77 d-k
ES-1	2.2 ab	7.8 a-e	2.6 a-d	0.76 b-h	2.2 a-d	7.7 a-e	2.5 a-f	0.76 f-k
ES-3	2.2 a-c	7.4 a-f	2.4 a-e	0.75 d-h	2.1 a-e	7.3 a-g	2.5 a-f	0.76 f-l
ES-2	2.0 a-e	7.0 a-g	2.1 a-g	0.79 a-f	2.1 a-e	7.5 a-f	2.7 a-d	0.80 b-i
C-22	2.2 a-c	8.0 a-d	2.5 a-d	0.84 ab	2.1 a-f	7.7 a-f	2.3 b-g	0.82 a-g
ES-6	2.1 a-e	7.6 a-f	2.4 a-e	0.84 ab	2.1 a-e	7.6 a-f	2.3 b-g	0.86 ab
Orange-14	2.1 a-e	7.4 a-f	2.2 a-f	0.78 b-h	2.1 a-e	7.4 a-f	2.2 b-g	0.79 c-j
UFR-4	2.1 a-d	8.0 a-c	2.5 a-d	0.80 a-e	1.9 b-h	6.8 c-i	1.9 d-i	0.83 a-f
UFR-5	2.1 a-e	7.5 a-f	2.2 a-g	0.76 b-h	2.0 a-g	7.0 b-h	2.1 b-g	0.75 g-l
US-897	2.0 a-e	7.2 a-f	2.1 a-g	0.75 d-h	2.0 a-g	7.1 b-h	2.1 b-g	0.78 d-k
ES-7	2.0 a-e	7.8 a-e	2.0 a-g	0.79 a-g	2.1 a-e	7.4 a-f	2.0 c-h	0.80 b-i
ES-5	2.0 a-e	7.4 a-f	1.8 a-g	0.86 a	2.2 a-d	7.2 a-h	2.1 c-g	0.83 a-e
Swingle	1.9 a-e	6.6 b-g	1.8 b-g	0.63 j	1.9 a-h	6.6 d-k	2.0 c-h	0.63 n
UFR-17	2.0 a-e	7.0 a-g	1.9 a-g	0.70 h-j	1.9 a-h	6.7 d-i	1.8 d-k	0.73 h-l
UFR-2	1.9 a-e	6.6 b-g	1.9 a-g	0.75 d-h	1.9 c-i	6.4 e-l	1.6 f-k	0.78 d-k
Green-7	2.1 a-e	7.3 a-f	2.1 a-g	0.71 g-i	1.9 b-h	6.3 f-l	1.4 g-k	0.71 k-m
ES-4	1.8 a-e	6.9 a-g	1.6 c-g	0.84 ab	2.0 a-g	7.1 b-h	1.9 d-j	0.85 a-c
UFR-1	1.8 a-e	6.5 b-g	1.6 c-g	0.75 d-h	1.9 a-h	6.5 d-l	1.8 d-k	0.78 c-k
White-1	1.7 a-e	6.3 d-g	1.6 c-g	0.73 e-h	1.8 d-j	6.6 d-k	1.7 d-k	0.76 e-k
Wgft+50-7	2.0 a-e	6.9 a-g	1.8 b-g	0.63 ij	1.9 c-i	6.0 g-l	1.3 g-k	0.69 l-n
UFR-6	1.8 a-e	6.3 c-g	1.5 d-g	0.81 a-d	1.8 d-j	6.8 c-i	1.6 e-k	0.84 a-d
Amb+Czo	1.8 a-e	6.9 a-g	1.5 d-g	0.84 ab	1.8 d-j	6.4 e-l	1.5 g-k	0.87 a
6058+2071-02-2	1.8 a-e	6.6 b-g	1.5 d-g	0.74 d-h	1.7 e-j	6.6 d-k	1.4 g-k	0.73 i-l
Sorp+Sh-991	1.8 a-e	6.5 c-g	1.5 d-g	0.71 f-i	1.4 ij	5.2 kl	0.9 jk	0.72 j-m
Amb+Benton	1.6 c-e	5.9 fg	1.2 e-g	0.82 a-d	1.6 g-j	5.8 h-l	1.0 h-k	0.82 a-g
UFR-3	1.6 b-e	5.9 fg	1.1 fg	0.71 h-j	1.6 f-j	5.1 l	0.9 i-k	0.65 mn
Green-3	1.5 e	5.3 g	1.0 g	0.72 e-h	1.5 h-j	5.4 j-l	1.0 i-k	0.73 i-l
Changsha+Benton	1.5 de	6.2 e-g	1.1 fg	0.83 a-c	1.4 j	5.5 i-l	0.8 k	0.88 a
Sour orange	2.0 a-e	8.0 a-d	2.0 a-g	0.86 a	—	—	—	—
F value	3.9***	6.19***	5.75***	15.38***	9.32***	12.17***	12.88***	19.95***

SRR, scion-to-rootstock trunk ratio. Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

highest for trees on 'ES-4' (59.1%) and lowest for trees on 'ES-7' (48.6%). 'Green-3', 'White-1', and sour orange induced the largest amount of TSS (9.40% to 9.80%), and 'C-22', 'C-146', and 'ES-2' induced the smallest amount of TSS (7.95% to 8.10%). The acid percentage was highest for trees on 'Green-3' (0.565%) and lowest for trees on 'ES-2' (0.443%). The TSS-to-acid ratio ranged from 16.2 to 18.4, but the mean separation was not significant.

At the Lake Wales location, the average fruit weight was 147 to 168 g, but there was no significant rootstock effect (Table 7). Juice percentage was highest for trees on 'Swingle' (58.3%) and lowest for trees on 'ES-7' (49.9%). 'UFR-5', 'Amb+Czo', and 'Changsha+Benton' induced the largest TSS % in the fruit (9.63% to 9.84%), whereas 'ES-2' and 'ES-3' induced the smallest TSS% (8.38% and 8.42%, respectively). 'UFR-5' induced the highest percentage of acid in the fruit (0.557%), and 'ES-4' and 'ES-6' induced the lowest (0.322% and 0.348%, respectively). The highest TSS-to-acid ratio was induced by 'ES-4' (28.4), and the lowest was induced by 'Swingle' (17.4). TSS was significantly and inversely correlated with canopy volume at the Basinger location (R = –0.68) and at the Lake Wales location (R = –0.53).

Leaf nutrient concentration

Leaf macronutrient (N, P, K, Ca, Mg, and S) and micronutrient (B, Zn, Mn, Fe, and Cu) concentrations were analyzed in production year 2019–20. The rootstock effect was significant for Mg and Mn at the Basinger location and for K, Mg, B, and Mn at the Lake Wales location (Supplemental Table 2).

At the Basinger location, the leaf Mg contents were highest for trees on 'ES-4' (0.40%) and lowest for trees on many of the other rootstocks (0.30% to 0.33%). Leaf Mn concentrations were significantly higher in trees on 'ES-3' (54 ppm) compared with trees on 'ES-6', 'Green-7', 'C-22', 'C-54', 'Changsha+Benton', and sour orange (38–40 ppm).

At the Lake Wales location, K concentrations ranged from 1.7% to 2.0%, but separation of means was not significant. Leaf Mg concentrations were highest for trees on 'X-639' (0.36%) and lowest for trees on 'ES-2' (0.29%). Leaf B concentrations were highest for trees on 'ES-7' (124 ppm) and lowest for trees on 'Amb+Benton' (101 ppm). The highest leaf Mn concentrations were induced by 'UFR-2' (69 ppm) and the lowest by 'UFR-17' and 'UFR-6' (49 and 50 ppm).

The average concentrations for other leaf nutrients were 3.1% (N), 0.19% (P), 1.9% (K), 2.5% (Ca), 0.32% (S), 98 ppm (B), 23 ppm (Zn), 71 ppm (Fe), and 178 ppm (Cu) at the Basinger location (data not shown). At the Lake Wales location, the average leaf nutrient concentrations were 2.9% (N), 0.17% (P), 2.7% (Ca), 0.33% (S), 35 ppm (Zn), 114 ppm (Fe), and 310 ppm (Cu) (data not shown).

Discussion

Considering the devastation caused by HLB, precocity may be one important criterion for rootstock selection to cope with economic losses due to tree decline when the disease reaches an advanced stage of progression. Our results showed significant variations among rootstocks in their effects on most of the horticultural traits measured. The identification of rootstock traits at an early stage of production is valuable for determining rootstock impacts on the economic viability of the mature orchard in the long term.

Although significant rootstock effects were measured, most trees had a healthy canopy with moderate foliar disease expression. The total percentage of dead trees was less than 2% in both locations, which is normal for young commercial citrus plantings. Trees on the small tree size-inducing rootstock 'Green-3' had the highest mortality rate among all rootstocks at the Basinger location. The low foliar disease expression of trees on this rootstock suggests that factors other than HLB may have been responsible.

The average percentages of wind-induced leaning of trees at both locations were 15.8% and 10.6% at Lake Wales and Basinger, respectively. The higher percentage of leaning trees at the Lake Wales location may be attributable to this location being part of the Central Florida Ridge, an ancient sand dune

Table 4. Fruit yield of 'Hamlin' orange trees on different rootstocks.

	Trial 1 (Basinger)		Trial 2 (Lake Wales)		
Rootstock	2018–19 (kg/tree)	2019–20 (kg/tree)	2018–19 (kg/tree)	2019–20 (kg/tree)	Avg cumulative (kg/tree)
ES-1	4.4 a	20.2 a	18.0 ab	16.3 a-e	29.5 a
UFR-5	6.7 a	17.4 a	20.2 a	11.8 a-h	28.0 ab
C-54	4.4 a	16.0 a	17.2 a-c	18.3 ab	28.0 ab
X-639	3.7 a	17.6 a	14.4 a-d	19.9 a	27.8 ab
C-57	7.3 a	14.7 a	13.4 a-d	17.7 a-c	26.6 a-c
ES-4	7.7 a	14.5 a	17.1 a-c	13.7 a-f	26.5 a-c
US-897	8.5 a	13.8 a	13.6 a-d	13.3 a-g	24.6 a-d
Swingle	7.2 a	15.6 a	13.9 a-d	12.1 a-h	24.4 a-e
ES-7	4.5 a	16.7 a	10.3 a-d	17.1 a-d	24.3 a-e
ES-6	5.1 a	18.9 a	11.4 a-d	11.9 a-h	23.6 a-e
ES-5	2.8 a	17.1 a	14.4 a-d	12.4 a-h	23.4 a-e
Green-7	7.3 a	19.9 a	10.5 a-d	9.0 d-h	23.2 a-e
C-22	6.0 a	13.3 a	13.2 a-d	13.7 a-f	23.1 a-e
Orange-14	7.6 a	13.2 a	12.0 a-d	13.2 a-h	22.9 a-e
ES-3	4.0 a	18.7 a	11.8 a-d	10.4 b-h	22.5 a-e
Amb+Czo	6.1 a	18.2 a	12.5 a-d	7.6 f-h	22.2 a-e
6058+2071-02-2	6.3 a	18.3 a	9.3 b-d	10.3 b-h	22.1 a-e
UFR-4	4.9 a	17.4 a	11.7 a-d	9.6 c-h	21.8 a-e
C-146	4.9 a	11.4 a	13.5 a-d	13.8 a-f	21.8 a-e
White-1	5.0 a	15.2 a	10.8 a-d	11.2 b-h	21.1 a-e
UFR-1	4.8 a	16.3 a	9.2 b-d	9.6 c-h	20.0 a-e
Wgft+50-7	6.1 a	18.2 a	6.0 d	9.5 c-h	19.9 a-e
UFR-17	4.5 a	15.6 a	10.9 a-d	8.4 e-h	19.7 a-e
UFR-6	6.8 a	13.8 a	10.1 a-d	7.1 f-h	18.9 a-e
UFR-2	6.3 a	16.4 a	7.7 cd	7.3 f-h	18.8 a-e
ES-2	3.9 a	11.2 a	11.1 a-d	10.8 b-h	18.5 a-e
Green-3	4.2 a	12.6 a	8.3 b-d	9.0 d-h	17.0 b-e
Amb+Benton	5.4 a	11.7 a	6.8 d	7.3 f-h	15.6 c-e
Changsha+Benton	6.5 a	10.5 a	9.0 b-d	4.6 h	15.3 c-e
Sorp+Sh-991	4.3 a	14.4 a	4.6 d	4.9 gh	14.1 de
UFR-3	4.9 a	9.6 a	6.2 d	5.7 f-h	13.1 e
Sour orange	7.4 a	13.3 a	—	—	—
F value	1.62*	1.78*	3.79***	6.19***	3.95***

Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 5. Yield efficiency of 'Hamlin' orange trees on different rootstocks.

	Trial 1 (Basinger)		Trial 2 (Lake Wales)		
Rootstock	2018–19 (kg/m^3)	2019–20 (kg/m^3)	2018–19 (kg/m^3)	2019–20 (kg/m^3)	Avg (kg/m^3)
Green-3	7.2 a	14.3 a	15.7 ab	10.5 a	11.9 a
Amb+Benton	9.9 a	12.5 a-c	11.5 a-d	7.9 ab	10.5 ab
Changsha+Benton	9.4 a	9.7 a-d	15.7 ab	6.0 ab	10.2 a-c
ES-4	7.9 a	9.7 a-d	15.0 a-c	7.6 ab	10.0 a-c
Amb+Czo	6.1 a	12.9 ab	13.4 a-d	5.8 ab	9.5 a-d
6058+2071-02-2	8.2 a	12.8 ab	7.6 b-d	7.4 ab	9.0 a-e
UFR-5	4.6 a	7.9 a-d	17.3 a	5.8 ab	8.9 a-e
Swingle	8.1 a	8.9 a-d	9.8 a-d	6.3 ab	8.3 a-f
UFR-3	6.5 a	8.4 a-d	11.3 a-d	6.5 ab	8.2 a-f
Green-7	6.1 a	9.5 a-d	10.7 a-d	6.4 ab	8.2 a-f
White-1	6.3 a	9.9 a-d	9.7 a-d	6.9 ab	8.2 a-f
UFR-6	8.0 a	9.9 a-d	9.8 a-d	4.7 b	8.1 a-f
Wgft+50-7	6.4 a	11.2 a-d	7.6 b-d	7.1 ab	8.0 a-f
Sorp+Sh-991	6.6 a	10.1 a-d	9.3 a-d	5.6 ab	7.9 a-f
UFR-1	5.8 a	10.5 a-d	7.9 b-d	5.6 ab	7.4 b-f
ES-1	3.6 a	8.3 a-d	11.1 a-d	6.5 ab	7.4 b-f
ES-7	4.2 a	8.2 a-d	7.9 b-d	9.0 ab	7.3 b-f
UFR-17	5.1 a	9.2 a-d	10.1 a-d	4.8 b	7.3 b-f
ES-5	2.8 a	10.5 a-d	9.7 a-d	6.2 ab	7.3 b-f
US-897	6.5 a	7.0 b-d	9.3 a-d	6.2 ab	7.3 b-f
Orange-14	7.1 a	6.0 b-d	7.5 b-d	6.0 ab	6.6 b-f
ES-6	4.1 a	8.2 a-d	9.1 a-d	5.1 b	6.6 b-f
UFR-4	3.0 a	7.3 a-d	10.3 a-d	5.1 b	6.4 b-f
C-22	4.5 a	5.4 d	9.0 a-d	6.3 ab	6.3 c-f
UFR-2	5.9 a	8.4 a-d	6.2 d	4.6 b	6.3 c-f
C-54	2.6 a	5.3 d	8.6 a-d	6.3 ab	5.7 d-f
C-57	4.3 a	5.8 b-d	6.1 d	5.8 ab	5.5 d-f
X-639	2.1 a	6.7 b-d	6.7 cd	6.0 ab	5.4 ef
ES-3	3.2 a	7.9 a-d	6.1 d	4.3 b	5.4 ef
C-146	3.3 a	4.3 d	8.0 b-d	5.3 ab	5.2 ef
ES-2	3.4 a	5.6 cd	6.1 d	4.2 b	4.8 f
Sour orange	7.2 a	6.7 b-d	—	—	—
F value	2.04**	3.54***	3.47***	1.87**	5.00***

Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 6. Fruit quality of 'Hamlin' orange trees on different rootstocks in trial 1 (Basinger).

Rootstock	Fruit wt (g)	Juice (%)	TSS (%)	Acid (%)	TSS-to-acid ratio
Green-3	174 a	57.2 a-d	9.80 a	0.565 a	17.4 a
White-1	162 a-f	56.6 a-d	9.53 ab	0.547 a-e	17.5 a
Sour orange	153 a-g	56.5 a-d	9.40 a-c	0.518 a-f	18.2 a
Changsha+Benton	169 ab	57.4 a-c	9.28 a-d	0.558 a-c	16.7 a
Wgft+50-7	171 ab	58.1 ab	9.27 a-d	0.562 ab	16.6 a
UFR-6	165 a-f	57.6 a-c	9.25 a-d	0.563 ab	16.5 a
Amb+Benton	167 a-d	57.0 a-d	9.23 a-e	0.547 a-e	16.9 a
UFR-1	158 a-g	57.1 a-d	9.18 a-f	0.543 a-e	16.9 a
Amb+Czo	164 a-f	57.1 a-d	9.14 a-g	0.548 a-d	16.7 a
Sorp+Sh-991	158 a-g	55.2 a-d	9.12 a-h	0.515 a-g	17.7 a
ES-4	161 a-g	59.1 a	9.11 a-h	0.520 a-f	17.5 a
Swingle	149 b-g	57.2 a-d	9.06 a-i	0.522 a-f	17.3 a
UFR-5	157 a-g	57.1 a-d	8.90 a-i	0.542 a-e	16.5 a
UFR-3	165 a-f	55.0 a-e	8.81 a-i	0.518 a-f	17.0 a
6058+2071-02-2	166 a-e	53.4 a-e	8.72 b-i	0.507 a-g	17.3 a
US-897	145 e-g	57.8 a-c	8.72 b-i	0.487 c-g	18.0 a
ES-5	161 a-g	52.8 a-e	8.69 b-i	0.473 e-g	18.4 a
Green-7	164 a-f	51.2 c-e	8.55 b-i	0.492 a-g	17.4 a
UFR-2	158 a-g	55.0 a-e	8.53 b-i	0.487 c-g	17.6 a
UFR-17	169 ab	53.7 a-e	8.49 b-i	0.515 a-g	16.5 a
ES-1	153 a-g	53.8 a-e	8.42 c-i	0.465 fg	18.1 a
C-54	146 c-g	52.4 b-e	8.35 c-i	0.458 fg	18.3 a
UFR-4	164 a-f	53.4 a-e	8.31 d-i	0.487 c-g	17.2 a
C-57	147 c-g	51.5 b-e	8.28 d-i	0.462 fg	18.0 a
ES-3	154 a-g	50.7 de	8.28 d-i	0.458 fg	18.1 a
ES-7	143 fg	48.6 e	8.28 d-i	0.457 fg	18.2 a
ES-6	168 a-c	51.7 b-e	8.27 d-i	0.463 fg	17.9 a
Orange-14	167 a-e	51.6 b-e	8.17 e-i	0.503 a-g	16.2 a
X-639	146 d-g	52.8 a-e	8.15 f-i	0.475 d-g	17.2 a
ES-2	140 g	51.5 b-e	8.10 g-i	0.443 g	18.3 a
C-146	149 b-g	51.7 b-e	8.08 hi	0.490 b-g	16.6 a
C-22	144 fg	50.5 de	7.95 i	0.465 fg	17.3 a
F value	5.59***	5.39***	6.50***	7.53***	2.01**

Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 7. Fruit quality of 'Hamlin' orange trees on different rootstocks in trial 2 (Lake Wales).

Rootstock	Fruit wt (g)	Juice (%)	TSS (%)	Acid (%)	TSS-to-acid ratio
UFR-5	153	57.2 a-e	9.84 a	0.557 a	17.7 ef
Amb+Czo	149	57.8 a-c	9.65 ab	0.505 a-g	19.1 c-f
Changsha+Benton	162	57.9 ab	9.63 ab	0.522 a-c	18.6 d-f
White-1	155	57.2 a-f	9.57 a-c	0.507 a-f	18.9 c-f
Green-3	157	55.0 a-g	9.47 a-d	0.510 a-e	18.6 d-f
UFR-1	149	57.4 a-d	9.47 a-d	0.515 a-d	18.4 d-f
Wgft+50-7	159	57.9 ab	9.46 a-d	0.537 ab	17.6 ef
Sorp+Sh-991	150	55.8 a-g	9.43 a-e	0.503 a-h	18.8 c-f
Amb+Benton	168	57.2 a-e	9.42 a-e	0.497 b-i	19.0 c-f
UFR-6	159	56.6 a-g	9.38 a-f	0.508 a-e	18.5 d-f
UFR-17	161	54.3 a-g	9.36 a-f	0.493 b-i	19.0 c-f
UFR-3	153	53.8 c-i	9.30 a-g	0.498 a-h	18.8 c-f
ES-5	151	54.2 b-g	9.29 a-g	0.477 c-j	19.5 c-f
Green-7	157	54.9 a-g	9.17 b-h	0.510 a-e	18.0 d-f
Swingle	150	58.3 a	9.16 b-h	0.526 a-c	17.4 f
Orange-14	157	55.9 a-g	9.09 b-h	0.500 a-h	18.2 d-f
US-897	148	58.0 ab	9.06 b-i	0.458 d-k	19.8 c-f
UFR-4	152	55.4 a-g	9.06 b-i	0.445 h-k	20.4 c-e
ES-4	156	54.9 a-g	9.05 b-i	0.322 l	28.4 a
C-22	154	53.1 g-i	8.97 c-j	0.432 jk	20.8 cd
UFR-2	157	55.2 a-g	8.88 d-j	0.478 b-j	18.6 d-f
C-54	149	55.7 a-g	8.83 d-j	0.460 d-k	19.3 c-f
ES-7	156	49.9 i	8.78 e-j	0.408 k	21.5 c
6058+2071-02-2	161	53.2 f-i	8.77 e-j	0.502 a-h	17.6 ef
C-146	159	53.6 d-i	8.74 f-j	0.438 i-k	20.0 c-f
X-639	155	54.0 b-h	8.68 g-j	0.452 e-k	19.2 c-f
ES-1	158	52.8 g-i	8.67 g-j	0.447 g-k	19.5 c-f
C-57	158	52.7 g-i	8.61 h-j	0.432 jk	20.0 c-f
ES-6	159	53.6 d-i	8.51 h-j	0.348 l	24.6 b
ES-3	151	50.0 hi	8.42 ij	0.448 f-k	18.8 c-f
ES-2	147	53.3 e-i	8.38 j	0.438 i-k	19.2 c-f
F value	1.15	8.97***	10.29***	21.65***	16.08***

Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

representing the highest elevation in Florida, and its characteristic sandy soils. Trees on some of the dwarfing rootstocks leaned more than trees on some of the larger size-inducing rootstocks, but the (inverse) correlation between tree size and leaning was weak. One of the exceptions was 'C-22', which induced a high percentage of leaning while producing larger than average trees. The leaning of trees did not appear to have a negative influence on tree health and productivity at the time of evaluation; however, it is expected that growers will prefer wind-tolerant rather than wind-susceptible cultivars. To our knowledge, this is the first report of citrus that compared rootstock cultivar effects on the ability of citrus trees to withstand tropical force winds. A study of apple rootstocks also found that trees on dwarfing rootstocks leaned more than trees on vigorous rootstocks (Schupp, 1992); this was attributed to the heavy crop load of dwarfing trees and weak anchorage. Differences in wind resistance among rootstocks observed in our study are likely associated with different root architectures because trees were bearing few fruit at the time of the hurricane. A study following the aftermath of hurricane Andrew, which destroyed much of the tropical fruit acreage in South Florida in 1992, found that the percentage of toppled and surviving trees was correlated with tree height and age and depended on the crop species and other factors (Crane et al., 1993).

The rootstocks induced a wide range of vigor in the 'Hamlin' scion. The diploid rootstocks produced mostly medium to large trees, with 'X-639', 'C-54', 'C-57', 'C-146', and 'ES-1' producing the largest trees in the trials. Surprisingly, 'US-897' and 'C-22', which are generally regarded as small tree size-inducing rootstocks (Bowman et al., 2008; Siebert et al., 2010), produced medium trees in both locations when compared with the rootstock standards 'Swingle' and sour orange which are known to produce medium trees. Other studies that included the rootstocks 'C-22', 'C-54', 'C-57', 'C-146', and 'X-639' showed similar results (Castle et al., 2011; Louzada et al., 2008; Roose, 2008). It is anticipated that the size-limiting effect of 'US-897' and 'C-22' will manifest during later production years. The delayed effect of 'US-897' on tree size has also been observed by commercial citrus growers (personal communications). It must be noted that some hybrids of *C. reticulata* and *P. trifoliata* (citrandarins) are regarded as HLB-tolerant (Albrecht and Bowman, 2012; Boava et al., 2014). A higher level of tolerance may have contributed to the vigorous growth of trees on the citrandarin rootstocks 'X-639', 'C-54', 'C-57', 'C-146', 'ES-1', 'US-897', and 'C-22' observed in our study.

Among the tetraploid rootstocks, 'Orange-14', 'UFR-4', and 'UFR-5' induced the largest tree size, which was average when compared across all rootstocks. Most of the other tetraploid rootstocks induced below-average size trees, which is in accordance with the results from other field trials conducted on both flatwood and Central Ridge sites in Florida (Grosser et al., 2011). During those trials, many of the tetraploid rootstocks induced a tree size smaller than 9 ft (2.7 m) after 10 years of growth. The dwarfing capacity of tetraploid rootstocks was suggested to be a graft union response because diploid and tetraploid cells differ morphologically, with the latter being larger and containing thicker cell walls (Grosser et al., 2011). However, the reduced growth of tetraploid rootstocks in comparison with diploid rootstocks was also observed at the seedling stage (Allario et al., 2011; Guerra et al., 2014). It is generally recognized that the reduced capacity of the dwarfing rootstocks to transport water from the soil to the aboveground part of the plant contributes to the vigor-inducing potential of a rootstock. Lower hydraulic conductivity was reported for apple trees on the dwarfing rootstock M.27 compared to those on the invigorating rootstock MM.106 (Atkinson et al., 2003). Forner-Giner et al. (2014) reported a reduced hydraulic conductance of 'Navelina' orange trees on dwarfing rootstocks 'FA-517' and 'FA-418' compared with trees on Carrizo rootstock. Factors such as soil and environmental conditions, management practices, pest and disease pressure, and rootstock compatibility with the scion may further affect the rootstock influence on tree size (Bowman and Joubert, 2020). The observed variations in the tree size induced at different locations for some of the rootstocks in our study were likely due to the differences in the soil and other environmental conditions because both sites had similar management practices, the same scion, and similar HLB disease pressure.

The SRR represents the smoothness of the graft union, and it has often been considered an indicator of the compatibility of scion and rootstocks (Kallsen and Parfitt, 2011). However, different vigor of the grafting partners can also result in overgrowth of one of the partners without posing any hazard to trunk health and tree physiology (Bowman and Joubert, 2020), as demonstrated by the long-time dominance of 'Swingle'. Together with 'Wgft+50-7', 'Swingle' induced the lowest SRR value in both trials.

The average yield at the Basinger location in 2018–19 was considerably lower than that at the Lake Wales location. One reason for this was an unusually high incidence of *Xanthomonas citri*, the causal organism of citrus canker, at the Basinger location, possibly as aftermath of hurricane Irma. Most of the high vigor-inducing rootstocks, such as 'X-639', 'ES-1', 'C-54', and 'C-57', were among the rootstocks that induced the highest cumulative yields across both production years and both locations, amounting to 149 to 158 boxes of fruit per acre (1 box = 90 lb or 40.8 kg of fruit). Although this amount is not yet considered commercially significant, many of the low vigor-inducing tetraploid rootstocks induced less than half this amount (70–83 boxes/acre), which, if this trend continues, mayprevent their commercial acceptance. Similar to our findings, in a rootstock trial with 'Marsh' grapefruit, 'X-639' and 'C-54' induced high cumulative yields and large tree sizes (Castle et al., 2011). In that trial, 'C-146' also produced high cumulative yields; however, our yield on this rootstock was average to low.

It is generally recognized that the size of the canopy is positively related to fruit yield (Anderson, 1987). An association between tree size and yield was also observed during rootstock trials with 'Valencia' and 'Hamlin' scion in Florida and with 'Pera' scion in Brazil (Albrecht et al., 2012; Bowman et al., 2016a; Quaggio et al., 2004). Despite the general relationship between canopy size and yield, in the present study, the medium vigor-inducing rootstock 'UFR-5' and the lower vigor-inducing 'ES-4' were among the rootstocks that induced higher than average cumulative yields. In contrast, the high vigor-inducing 'ES-2' rootstock induced a lower-than-average cumulative yield. The correlation between canopy size and yield in our trials was moderate (0.39) to high (0.66), depending on the location, suggesting that the canopy size of a citrus tree may not always be a good predictor of yield.

High yield-inducing rootstocks do not necessarily increase the productivity of a citrus orchard if the planting density is not optimized for the size of the mature productive tree (Bowman and Joubert, 2020). Hence, yield efficiency (yield per unit canopy volume) is a more suitable variable for determining productivity of a mature orchard. In two recent studies of 'Ray Ruby' grapefruit, high-density planting resulted in increased yield per hectare, increased fruit TSS contents, and decreased canopy volume (Phuyal et al., 2020). This demonstrates that the use of high-yield efficient rootstocks and optimized planting density can increase productivity and minimize cost per unit production.

Most of the high vigor-inducing rootstocks, such as 'X-639', 'C-54', 'C-57', and 'C-146', and some of the medium vigor-inducing rootstocks, such as 'ES-2' and 'ES-3', were not as yield-efficient in our study as the lower vigor-inducing rootstocks. Among the rootstocks that induced the highest yield efficiency were the tetraploid rootstocks 'Green-3', 'Amb+Benton', 'Changsha+Benton', 'Amb+Czo', and '6058+2071-02-2', which produced some of the smallest trees in the trials. However, despite having high yield efficiency, the overall yield potential of these rootstocks may not be sufficient, in the longer-term, to compete with the more vigorous rootstocks. Moreover, some of these small size-inducing rootstocks were leaning because of the strong winds imposed by the hurricane in 2017; the long-term impact remains to be investigated. 'ES-4', 'UFR-5', and 'Swingle' were among the rootstocks inducing higher-than-average yield efficiency across both locations and production years while producing medium-sized trees.

The priority for different fruit quality traits varies in different production areas based on the scion used and purpose (fresh

fruit or juice production). For sweet oranges like 'Hamlin' that are grown for juice processing, TSS (Brix) is the most important fruit quality variable, followed by the acid content and the ratio of the two. Similar to other citrus studies, rootstocks in this study induced significant differences in all measured fruit quality variables in both trials (Bowman et al., 2016b; Grosser et al., 2011; McCollum and Bowman, 2017).

Most rootstocks induced more than 50% juice, except for 'ES-7', which is a lemon-type hybrid. Despite differences among locations for some of the rootstocks, there was a tendency for dwarfing and semi-dwarfing rootstocks to induce larger amounts of TSS than vigorous rootstocks. The inverse correlation between tree size and TSS was more evident at the Basinger location (R = –0.78) than at the Lake Wales location (R = –0.53). Among the highest TSS-inducing rootstocks in both locations were the smallest size-inducing tetraploid rootstocks 'Green-3', 'White-1', 'Changsha+Benton', 'Amb+Benton', 'Amb+Czo', 'Wgft+50-7', 'UFR-1', and 'UFR-6'. These rootstocks were also found to induce excellent fruit quality during a previous field trial with 'Valquarius' scion (Grosser et al., 2011). The low vigor-inducing rootstock 'US-897' and the high vigor-inducing rootstock 'X-639' induced the highest and lowest TSS contents, respectively, during a study of 'Ray Ruby' grapefruit trees (McCollum and Bowman, 2017).

The influence of rootstocks on fruit quality is assumed to be due to the different roles of rootstocks in the translocation of photoassimilates to the fruits (Gardner, 1969) and the movement of water and sucrose to the juice sacs (Castle, 1995). Because these factors also affect the vegetative growth of a tree, the inverse correlation found between TSS and the vigor-inducing capacity of rootstock is expected. In addition to rootstock cultivar, climatic factors (Davies, 1997) and diseases such as HLB can affect the citrus fruit quality (Baldwin et al., 2018; Liao and Burns, 2012) and interact with the rootstock (Dala-Paula et al., 2019). Two of the rootstocks ('ES-4' and 'ES-6') at the Lake Wales location induced a very low acid content, thereby increasing the TSS-to-acid ratio to more than 24. In general, acid ratios were very low in the 2019–20 production season. Unusually high temperatures and excessive rainfall throughout the year, particularly during the preharvest period, may have been responsible for this. The negative influence of warm temperatures on TSS and acid content was previously highlighted (Reuther, 1980; Zekri, 2011).

It is accepted that rootstocks differ in their capacity to uptake nutrients from the soil and distribute them through the scion (Brown et al., 1994; Toplu et al., 2012; Wutscher, 1973). In our study, the rootstock effect was not significant for most of the leaf nutrients and not consistent across both trials. Overall, most of the nutrients were in the range deemed optimal or high for citrus (Kadyampakeni and Morgan, 2020). The high Cu concentration measured in trees at both locations are the result of frequent Cu applications to control citrus canker, which is widespread in Florida (Dewdney et al., 2001; Zambon et al., 2019).

Conclusions

The diploid and tetraploid rootstocks investigated during this study differed in their effects on the 'Hamlin' scion. Among the most prominent effects were those on tree size and productivity. The early production of high-quality fruits and high productivity comprise one strategy that can result in growing citrus profitably under HLB-endemic conditions. Many of the tetraploid rootstocks reduced tree size significantly and consequently reduced yield; however, they increased yield efficiency. Therefore, these rootstocks may be suitable for high-density plantings to increase orchard productivity during the early production years. However, many of the small size-inducing rootstocks rendered the trees more vulnerable to tropical force winds than the large size-inducing rootstocks. Although this study was conducted during the early production years, the results are valuable for predicting rootstock resilience and their economic potential in the longer term.

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Horticultural Attributes and Root Architectures of Field-grown 'Valencia' Trees Grafted on Different Rootstocks Propagated by Seed, Cuttings, and Tissue Culture

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***Abstract*. Huanglongbing (HLB) is a devastating disease of citrus that is found in most citrus production areas around the world. The bacterium associated with HLB resides in and damages the phloem, restricting the movement of photosynthates throughout the plant and leading to tree decline. Considerable root loss can be observed in affected trees even when few disease symptoms are visible aboveground. Root traits can substantially influence tree performance and use of superior rootstocks is one strategy to manage tree health and reduce production losses in a disease-endemic environment. Citrus rootstocks are typically propagated by seed, but due to the increased demand for some of the best-performing cultivars, propagation by other methods is being used to overcome seed shortages. In this research, differences in root architecture and root growth of six different rootstocks propagated by seed, cuttings, and tissue culture, and their influence on the grafted 'Valencia' (*Citrus sinensis*) scion were investigated. A field trial was established in southwest Florida in 2017. Trees were evaluated for their performance during the first 2 years after planting and a subset of trees was excavated for detailed analysis of root architectures and biomass distribution. Significant differences among propagation methods were found for the rootstock trunk diameter and the lateral (structural) root length, which were largest in seed-propagated rootstocks. Most of the other horticultural and root architectural traits were not significantly influenced by the rootstock-propagation method; however, many of the measured variables were significantly influenced by the rootstock cultivar regardless of the propagation method. The results showed that rootstocks propagated by cuttings and tissue culture were similar to seed-propagated rootstocks in their influence on the grafted tree during the early years of growth in the field.**

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Until the 1800s, the typical method of citrus propagation was by growing seed of the desired fruiting variety (Castle, 2010). Although grafting in citrus has been reported since the Roman era, the commercial cultivation of grafted citrus did not commence until the 19th century (Mudge et al., 2009). The most used rootstocks for grafting before the 1970s were sour orange (*Citrus aurantium*) and rough lemon (*Citrus ×jambhiri*). As the importance of rootstocks for citrus production was recognized, other rootstocks came into use (Castle, 2010). Historically, major disease events in citrus such as gummosis (1834), phytophthora root rot (1842), and citrus tristeza virus (1930s), which decimated citrus production on a large scale, were managed by using disease-resistant or tolerant rootstock cultivars (Bitters, 1986; Bowman and Joubert, 2020). The rootstock also plays an important role in reducing plant juvenility, inducing tolerance to various abiotic and biotic stresses, and enhancing the horticultural performance of the tree (Bowman and Joubert, 2020).

HLB, also known as citrus greening, is one of the most devastating diseases of citrus, and affects citrus production worldwide (Bove, 2006; McCollum and Baldwin, 2017). Since the discovery of HLB in Florida in 2005, citrus production has declined from 13 million tons in 2003–04 to 3.5 million tons in 2018–19 (www.nass.usda.gov/fl). With no cure available, various management strategies are used to reduce the negative impacts of the disease on trees. HLB is associated with nonculturable bacteria of the genus *Liberibacter*, among which *Candidatus* Liberibacter asiaticus is the most prevalent species. Control of the HLB vector, the Asian citrus psyllid, is crucial to prevent infection, and insecticide applications (Qureshi et al., 2014) and introduction of natural predators (Qureshi et al., 2009), are common practice. To maintain tree productivity and longevity in HLB-affected groves, enhanced application of micronutrients and irrigation management are included for an integrated disease management (Morgan et al., 2016; Stansly et al., 2014; Xia et al., 2018).

Although most commercial citrus scion cultivars are susceptible (McClean and Schwarz, 1970), several rootstock cultivars are tolerant to HLB (Albrecht and Bowman, 2011, 2012; Folimonova et al., 2009; Ramadugu et al., 2016). The exact mechanisms for the beneficial influence of rootstocks on the scion are unclear. Root traits such as the root system architecture, root size distribution, and the regeneration capacity of fibrous roots are important attributes that were shown to influence tree growth and tolerance to biotic and abiotic stresses (Atucha et al., 2014; Freeland, 2016; Graham 1995). The suggested mechanisms influencing tree vigor are related to the xylem vessel anatomy and the hydraulic conductivity of the rootstock (Forner-Giner et al., 2014; Martínez-Alcántara et al., 2013; Vasconcellos and Castle, 1994).

With the endemic presence of HLB in Florida, rootstocks have gained renewed interest because they provide a tool to manage HLB at no additional cost. Although the degree by which HLB-tolerant rootstocks can influence the grafted tree tolerance is not sufficient to completely suppress the disease, some rootstocks can significantly improve tree performance and productivity in an HLB-endemic environment (Boava et al., 2015; Bowman et al., 2016a, 2016b; Bowman and McCollum, 2015; Shokrollah et al., 2011).

The phenomenon of nucellar embryony in citrus allows the true-to-type production of plants from the seeds of many citrus species (Koltunow et al., 1995), including all rootstocks of commercial importance. However, because of the high demand for new

HLB-tolerant rootstocks, there is a shortage of seeds for some of the most desired cultivars (Albrecht, et al., 2020). In addition, although nucellar embryony is common, not all cultivars exhibit this trait and some outstanding new rootstock cultivars may be unsuitable for seed propagation (Bisi et al., 2020). For these reasons, it is valuable to also make use of alternative propagation methods such as cuttings and tissue culture to produce genetically identical rootstocks that can be used as liners for grafting (Albrecht et al., 2017a).

Based on traditional views, some growers and nursery owners are reluctant to use cuttings- and tissue culture–propagated rootstocks because they perceive them as inferior to seedling rootstocks. One of the main concerns is the different root system architecture of vegetative propagated rootstocks, particularly the absence of a taproot. During the nursery stage, seed-propagated rootstocks usually have a well-defined single tap root system, whereas cuttings- and tissue culture–propagated rootstocks have an adventitious root system with multiple smaller-diameter roots (Albrecht et al., 2017a, 2020). It is generally assumed that the lack of a tap root system can render trees more susceptible to wind-induced uprooting, which is of concern in Florida where tropical storms and hurricanes are an annual threat (Castle, 1977; Crane et al., 1993).

Fibrous roots are responsible for the uptake of water and nutrients from the soil. It was suggested that HLB significantly affects the citrus root system and causes fibrous root loss before manifestation of disease symptoms in the tree canopy (Johnson et al., 2013; Kumar and Kiran, 2018). Therefore, a well-structured root system with healthy fibrous roots is likely to be beneficial in an HLB-endemic environment.

It is well documented that different rootstock cultivars exhibit differences in their root architecture (Albrecht et al., 2020; Castle and Youtsey, 1977; Eissenstat, 1991) that contribute to their influence on the aboveground horticultural traits (Bowman et al., 2016a, 2016b; Bowman and Joubert, 2020) and may interact with the propagation method. In our laboratory, we recently investigated root architectures of rootstock liners and grafted trees during the nursery stage (Albrecht et al., 2017a, 2020). Here we examined in detail the root growth and root architectures of 'Valencia' orange (*Citrus sinensis*) trees grafted on different rootstocks propagated by seed, cuttings, and tissue culture, and their influence on aboveground horticultural traits after 2 years of growth in a southwest Florida field environment. The objective was to determine whether the method of propagation influences tree growth in an open field setting and whether the rootstock cultivar is an interacting factor.

Materials and Methods

Plant material

Rootstock cultivars. Six commercial rootstock cultivars were used: 'US-802' ['Siamese' pummelo (*Citrus maxima*) × 'Gotha Road' trifoliate orange (*Poncirus trifoliata*)], 'US-897' ['Cleopatra' mandarin (*Citrus reticulata*) × 'Flying Dragon' trifoliate orange], 'US-812' ['Sunki' mandarin (*C. reticulata*) × 'Benecke' trifoliate orange], 'US-942' ('Sunki' mandarin × 'Flying Dragon' trifoliate orange), 'Swingle' ['Duncan' grapefruit (*Citrus ×paradisi*) × trifoliate orange], and 'US-1516' ['African' pummelo (*Citrus maxima*) × 'Flying Dragon' trifoliate orange]. These rootstocks were among the top 15 most propagated rootstocks in Florida during the most recent production years (Florida Department of Agriculture and Consumer Services, 2020). All rootstocks were propagated by seed, by cuttings, or by tissue culture as described below. Plants were grown in the U.S. Department of Agriculture Horticultural Research Laboratory (USHRL) greenhouses in Fort Pierce, FL.

Seed propagation. Seeds were extracted from fruits as described in Albrecht et al. (2017a). Seeds were sown into premoistened soilless potting mix (Pro Mix BX; Premier Horticulture, Inc., Quakertown, PA) contained in racks of 3.8 cm × 21 cm cone cells (Cone-tainers; Stuewe and Sons, Tangent, OR). After germination, any off-types arising from zygotic embryos were identified based on their different leaf morphological traits and discarded. Plants were irrigated by hand as needed and fertilized biweekly using a water-soluble fertilizer with micronutrients (20N–10P–20K; Peters Professional, The Scotts Company, Marysville, OH) at a rate of 400 mg N/L. Insecticides were applied as needed.

Cuttings propagation. Cuttings were prepared following the method described in Bowman and Albrecht (2017). Single-node cuttings were excised from woody sections of 1- to 2-year-old greenhouse-grown nucellar seedlings. The leaves on each node were trimmed to reduce the leaf area to 20% to 30%. The basal end of each cutting was dipped in a commercial rooting powder (Hormodin 2; E.C. Geiger, Inc., Harleysville, PA) containing 0.3% indole-3-butyric acid (IBA). Cuttings were inserted into premoistened potting medium in cone cells as described for seed propagation and placed on a mist bench. Misting was applied for a duration of 6 weeks under shading from 9:00 AM to 6:00 PM daily. Liquid fertilizer was applied after 4 weeks as described previously, and again after 2 weeks, at which time chelated iron (Sequestrene 138 Fe; Ciba-Geigy Corp., Greensboro, NC) was applied simultaneously. After 7 weeks, plants were maintained in the same manner described for seedlings.

Tissue culture propagation. The source of explants for tissue culture–propagated plants were nucellar embryos from seeds obtained from fruit collected from foundation trees at the Bureau of Citrus Budwood Registration, Florida Department of Agriculture and Consumer Services, Chiefland, FL. Tissue culture propagation followed the procedure described in Albrecht et al. (2020). Disinfected embryos were placed into clear polypropylene containers containing Murashige and Skoog (MS) agar-nutrient medium (Murashige and Skoog, 1962) without added growth regulators. Embryos were dissected after pre-germination of seeds and identified as nucellar based on leaf morphology and uniformity of growth of the regenerated plant. Multiple shoot clusters were produced by alternating between media containing MS medium with 1.0 mg/L benzyladenine (BA), 0.5 mg/L kinetin, and 0.5 mg/L naphthalene acetic acid (NAA) (Bowman et al., 1997), and MS medium or EXS-III basal medium (PhytoTechnology Laboratories, Lenexa, KS) with no added growth regulators. Clusters were divided and placed in new media on a cycle of ≈5 weeks. Elongated shoots were produced by serial transfers on hormone-free medium and single shoots with at least four nodes were excised and placed on MS basal medium containing 2.0 mg/L NAA and 1 g/L active charcoal. After rooting for ≈6 weeks, plantlets were removed from the medium, roots were trimmed to 3 to 6 cm length, and placed into cone cells with premoistened potting medium, as described for seed propagation. Plants were kept in high humidity in a plant growth chamber (EGC Model M36; Environmental Growth Chambers, Chagrin Falls, OH) with a 16 h light/8 h darkness photoperiod. Humidity was gradually reduced over 3 to 4 weeks and plants were transferred to the greenhouse and maintained as described for seedlings.

Grafting. When rootstock liners were of a suitable size (4–6 mm stem diameter), they were transplanted into 15.2 cm × 15.2 cm × 30.5 cm plastic treepots (Stuewe & Sons) containing Pro Mix BX potting medium. After an acclimatization period, liners were budded with certified disease-free 'Valencia' orange budwood using the inverted T method (Albrecht et al., 2017b). Plants remained under natural light conditions and were irrigated as needed. Fertilization occurred biweekly as described previously and insecticides were applied as needed.

Experimental design

The field trial was established in Nov. 2017 at the Southwest Florida Research and Education Center in Immokalee, FL (Collier County, 26.462996, –81.443710). Grafted trees were planted in two, double-row beds separated by furrows. The distance between rows was 22 ft (6.7 m) and trees in each row were spaced at 4 ft (1.2 m). Trees were arranged in a randomized split plot design with rootstock cultivar as the main plot factor and propagation method as the subplot factor. Four replications (one per row) were included in the trial with each subplot consisting of four trees. Two border trees were planted at the end of each row. The trees were maintained according to commercial grower standards with regular root drenches of neonicotinoids, foliar sprays of other insecticides, and weed management, as needed. Irrigation was by under-tree microjets. Granular and slow-release fertilizer (12N–3P–9K; Harrell's fertilizer, Lakeland FL) was applied

at a rate of 1 pound (0.45 kg) per tree, three times per year (spring, summer, and fall).

The soil type at this location is a sandy spodosol of the Immokalee series with little organic matter, low cation exchange capacity, poor drainage, and low water-holding capacity (Mylavarapu et al., 2016). At the end of the trial, random soil samples were collected across each row to a depth of 25 cm near the canopy drip line and pooled for physicochemical analysis (Waters Agricultural Laboratories, Inc., Camilla, GA). Soil analysis found an organic matter content of 0.47%, a pH of 7.53, and a cation exchange capacity of 4.25 meq/100 g. Sand, silt, and clay contents were 94.74%, 3%, and 2.1%, respectively.

Plant assessments

Horticultural attributes. Aboveground horticultural attributes were evaluated 2 years (Nov. 2019) after planting. Tree height was measured from the soil surface to the top of the canopy (excluding any erratic shoots) using a measuring tape. Canopy spread was measured in two cardinal directions and canopy diameter was expressed as the average of the two measures. Canopy volume was calculated using the formula described in Wutscher and Hill (1995), given as follows: canopy volume = (diameter^2 × height)/4. Scion and rootstock trunk diameters were measured at 5 cm above and below the graft union using a digital caliper (Mitutoyo America, Aurora, IL). Two measurements were taken perpendicular to one another, and averages were determined.

Canopy health and foliar disease symptoms indices. Canopy health (canopy color and canopy thickness) and foliar disease symptoms were assessed in Oct. 2019 by visual ratings. Canopy color and canopy thickness were rated on a scale of 1 to 5, with 1 representing the worst (very yellow unhealthy canopy; very thin canopy) and 5 representing the best (very healthy dark green canopy; very thick canopy). Foliar HLB disease symptoms were rated on a scale of 1 to 5, with 1 representing the best (no symptoms) and 5 representing the worst (75% to 100% of the canopy displaying symptoms); ratings of 2, 3, and 4 represented 1% to 25%, 25% to 50%, and 50% to 75% of affected canopy, respectively. Two ratings on opposite sides of each tree were conducted and expressed as averages.

DNA extraction and Candidatus *Liberibacter asiaticus (CLas) detection.* Ten mature leaves were randomly collected from each tree in Oct. 2019 and leaves were pooled within a subplot. Midribs and petioles were excised and pulverized in liquid nitrogen using a mortar and pestle. One hundred milligrams of ground sample was used for DNA extraction. DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, MD) according to the manufacturer's instructions. Quantitative real-time polymerase chain reaction (qPCR) was conducted using a CFX96 Real-Time PCR detection system (Bio-Rad, Hercules, CA). PCR detection of CLas was conducted as described in Albrecht and Bowman (2019) using primers HLBas/HLBr and probe HLBp (Li et al., 2006); primers Coxf/Coxr and probe Coxp were used for assessing the quality of DNA extraction and for normalization.

Leaf nutrient analysis. Leaf nutrients were analyzed in July 2018 and 2019. Mature leaves from the recent spring flush were randomly collected from each tree within each subplot and pooled for a total of 30 leaves per sample. Analysis of macro- (N, P, K, Ca, Mg, S) and micronutrients (B, Zn, Mn, Fe, Cu) was conducted by Waters Agricultural Laboratories, Inc. The total nitrogen content was determined by the combustion method described in Sweeney (1989). The other macro- and micronutrients were analyzed using inductively coupled argon plasma atomic emission spectroscopy (Havlin and Soltanpour, 1980; Huang and Schulte, 1985) after digesting leaves with nitric acid and hydrogen peroxide.

Root growth measurement. To study the root growth, 6 cm × 50-cm clear acrylic minirhizotron tubes (CID BioScience, Camas, WA) were inserted next to a subset of trees immediately after planting. The tubes, which were closed on the bottom, were inserted into the soil at an angle of 45° with the soil surface and 45 cm from the tree trunk. A black foam core was placed in each tube and the portion of each tube protruding from the soil was covered with a black plastic bag to prevent sunlight and water from entering the tubes. Every second plant in each subplot received one minirhizotron for a total of 144 tubes. Root images were captured monthly starting in Dec. 2017. Images were captured with a CI-600 in situ root imaging system (CID Bio-Science). Each image represented a 360-degree view of the root zone facing the tube and was 30.5 × 20.0 cm in size. Two images were taken per minirhizotron, which represented the top 33 cm of the soil. Root images were analyzed using WinRhizoTRON software (Regent Instruments Inc., Quebec, Canada). Root growth was measured as the sum of roots visible in the imaging area and expressed in centimeters; live and dead roots were distinguished by their color and structural integrity. Growth was measured for 1 year, during which time there was no overlap of roots from adjacent trees.

Root architecture and biomass distribution

Tree excavation. Two years after planting, one tree per subplot (72 trees total) was excavated for a detailed evaluation of root architectures and aboveground and belowground biomass distribution. Tree excavation was performed using a pneumatic arborist tool (2000 Model HT142; Airspade, Chicopee, MA) and compressed air. This allowed the excavation of the whole root system without damage or loss of fibrous roots.

Scion biomass and leaf area. Trees were cut at the graft union using a pruning saw. Leaves were removed, and a random subsample of 30 mature leaves was used for leaf area determination. Leaf subsamples were scanned on a flatbed scanner (Epson perfection V850; Epson America Inc., Long Beach, CA) at 300 dpi, and the leaf area was measured using Assess 2.0 software (The American Phytopathological Society, St. Paul, MN). Leaves and the remaining scion were placed in paper bags, oven dried at 49 °C until constant weight, and weighed. The specific leaf area (SLA) was determined as the ratio of the leaf area (m^2) to the leaf dry weight (g). The total leaf area of the tree was calculated by multiplying SLA with the total leaf dry mass of the tree.

Rootstock biomass and root architecture. After excavation, the lateral (structural) roots (>2 mm in diameter) were cut off at 5 inches (13 cm) from the center of the root crown. Root system depth was measured from the soil level to the depth of the most distal roots on the root crown. Lateral roots were counted, and root diameters were measured at the point of separation from the root crown using a digital caliper (Mitutoyo America, Aurora, IL). Fibrous roots (<2 mm in diameter) were separated from the lateral roots, and a subset was used for determination of the specific root length (SRL). Roots were scanned on a flatbed scanner (Epson Perfection V850) at 400 dpi and the total root length was measured using Assess 2.0. Root crowns (including the rootstock trunk portion below the graft union), lateral roots, and fibrous roots were dried and weighed as described previously. The SRL was determined as the ratio of the fibrous root length (m) to its dry weight (g).

Statistical analysis

Analysis of variance (ANOVA) was conducted using R version 3.6.2 (R Core Team, Vienna, Austria, 2019) for all variables. A linear mixed model was used for ANOVA with block as a random factor and rootstock and method of propagation as fixed factors. Monthly root growth was analyzed using repeated measures ANOVA. Mean separation was performed by Tukey's honestly significant difference test. Differences were defined as statistically significant when the P value was < 0.05.

Results

Horticultural attributes and canopy health. After 2 years of field growth, trees were 128 to 131 cm tall with a canopy volume of 0.53 to 0.60 m^3 and a scion trunk diameter of 3.7 to 3.9 cm, but none of these variables were influenced by the rootstock-propagation method (Table 1). In contrast, the rootstock trunk diameter was significantly larger (5.6 cm) for rootstocks propagated by seed than by cuttings (5.2 cm). The scion to rootstock trunk diameter ratio was significantly different among trees, with trees on cuttings-propagated rootstocks having the highest ratio (0.75).

All horticultural attributes measured were significantly influenced by the rootstock cultivar. Trees on 'US-897' were shortest (119 cm) and had the smallest canopy volume

Table 1. Horticultural attributes of 'Valencia' trees grafted on different rootstocks propagated by seed (SD), cuttings (CT), and tissue culture (TC).

Factor	Plant ht (cm)	Canopy vol (m^3)	Scion trunk diam (cm)	Rootstock trunk diam (cm)	Scion to rootstock trunk diam ratio
Propagation method					
SD	131	0.60	3.9	5.6 a	0.68 b
CT	130	0.58	3.9	5.2 b	0.75 a
TC	128	0.53	3.7	5.3 ab	0.71 b
P value	0.6024	0.2061	0.1940	0.0185*	<0.0001***
Rootstock					
Swingle	129 ab	0.54 ab	3.8 abc	5.8 a	0.65 c
US-1516	134 a	0.54 ab	3.8 abc	5.5 a	0.70 b
US-802	131 a	0.57 ab	3.7 bc	5.7 a	0.65 c
US-812	135 a	0.61 ab	4.1 ab	5.4 a	0.76 a
US-897	119 b	0.46 b	3.4 c	4.5 b	0.76 a
US-942	137 a	0.70 a	4.3 a	5.4 a	0.80 a
P value	0.0040**	0.0397*	0.0026**	0.0008***	<0.0001***
Propagation method × rootstock					
P value	0.5458	0.2308	0.1725	0.1196	0.1030
Block					
P value	0.0001***	0.0002***	0.0198*	0.0207*	0.1960
Block × rootstock					
P value	0.9999	0.4871	0.1720	0.5087	0.5080

Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. *, **, *** denote *P* values significant at 5%, 1%, and less than 0.1% level of significance.

Table 2. Canopy health and foliar Huanglongbing (HLB) disease symptom indices and leaf threshold cycle (Ct) values of 'Valencia' trees grafted on different rootstocks propagated by seed (SD), cuttings (CT), and tissue culture (TC).

Factors	Canopy thickness	Canopy color	Foliar disease symptoms	Ct values
Propagation method				
SD	3.5	3.3	2.53	23.3
CT	3.4	3.4	2.43	23.4
TC	3.2	3.3	2.50	23.3
P value	0.1129	0.4269	0.5325	0.8565
Rootstock				
Swingle	3.1 b	3.3	2.47 ab	23.3
US-1516	3.3 ab	3.4	2.38 ab	23.1
US-802	3.2 b	3.2	2.66 ab	23.3
US-812	3.5 ab	3.4	2.44 ab	23.0
US-897	3.5 ab	3.2	2.68 a	22.9
US-942	3.7 a	3.5	2.29 b	24.1
P value	0.0029**	0.1406	0.0231*	0.3138
Propagation method × rootstock				
P value	0.7046	0.6296	0.7502	0.7038
Block				
P value	0.0075**	0.1121	0.0693	0.3832
Block × rootstock				
P value	0.9999	0.9999	0.9999	0.0005***

Indices were determined by ratings on a scale of 1 to 5, with 1 being the worst and 5 being the best for canopy thickness and canopy color and 1 being the best and 5 being the worst for foliar HLB symptoms. Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. *, **, *** denote *P* values significant at 5%, 1%, and less than 0.1% level of significance.

(0.46 m^3) and scion (3.4 cm) and rootstock (4.5 cm) trunk diameter. Trees on 'US-942' had the largest canopy volume (0.70 m^3) and scion trunk diameter (4.3 cm). Trees on 'US-942' also had the largest scion to rootstock trunk diameter ratio along with 'US-812'and 'US-897'. The smallest scion to rootstock trunk diameter ratio was found for trees on 'Swingle' and 'US-802'.

No significant interaction between propagation method and rootstock cultivar was observed for plant height, canopy volume, scion trunk diameter, rootstock trunk diameter, and scion to rootstock trunk diameter ratio. Block was found significant for all traits except scion to rootstock diameter ratio.

Canopy thickness, canopy color, and foliar disease symptom indices were not significantly different among trees on rootstocks propagated by the different methods (Table 2). Canopy thickness was significantly influenced by the rootstock cultivar. Trees on 'US-942' had the thickest canopy (3.7) whereas trees on 'Swingle' and 'US-802' had the thinnest canopy (3.1 and 3.2). Rootstock cultivar did not significantly influence canopy color but influenced the foliar disease symptom expression. Trees on 'US-897' had the highest (2.7) disease symptom index and trees on 'US-942' had the lowest (2.3). No significant interaction was found between propagation method and rootstock cultivar or between block and rootstock cultivar for canopy thickness, canopy color, and foliar HLB symptoms.

All subplots tested positive for CLas. The average threshold cycle (Ct) value was 23.3, and was not influenced by propagation method, rootstock cultivar, or their interaction (Table 2). There was a significant interaction between block and rootstock cultivar.

Leaf nutrients. Leaf nutrient concentrations analyzed in 2018 and 2019 were not significantly influenced by the rootstock-propagation methods, except for calcium (Ca) (Supplemental Tables 1 and 2). Leaf Ca concentrations were higher (3.0% and 3.1%) on seed and cuttings-propagated rootstocks and lower (2.9%) on tissue culture–propagated rootstocks, but only in 2019.

Rootstock cultivar had a significant influence on the concentrations of some of the leaf macro- and micronutrients. Leaf potassium (K), zinc (Zn), and manganese (Mn) concentrations were significantly influenced by rootstock cultivar in 2018 (Supplemental Table 1). Concentrations of Zn and Mn were highest (62 ppm and 978 ppm) in trees on 'US-802' and lowest (46 ppm and 714 ppm) in trees on 'US-942'; post hoc separation of means for K was not significant. A significant interaction between rootstock-propagation method and cultivar was observed for Ca and B in 2018. Leaf B content was highest (184 ppm) on 'US-802' propagated by

cuttings and lowest (109 ppm) on 'US-812' propagated by seed.

In 2019, significant differences were observed among trees on different rootstocks for the concentrations of K and Ca (Supplemental Table 2). Trees on 'Swingle' and 'US-1516' had the highest (1.6%) K concentration and trees on 'US-897' had the lowest (1.4%). Similarly, calcium concentration was highest (3.1%) on 'US-942' and lowest (2.6%) on 'Swingle'. An interaction with propagation method was found for Cu, which was higher in concentration (40 ppm) in trees on 'Swingle' propagated by cuttings than in any other combination of rootstock and propagation method.

Root growth. The total growth of roots during the first year in the field and the percentage of live and dead roots assessed by minirhizotron image analysis is shown in Table 3. The total root length at the end of year 1 was 1.3 to 1.7 m and did not differ among propagation methods or rootstock cultivars, but block was a significant factor. There were also no significant differences found for the live root length. The percentage of dead roots was not influenced by rootstock-propagation method but was significantly influenced by rootstock cultivar. 'US-942' had the highest (65%) percentage of dead roots, whereas 'US-1516' had the lowest (30%). No interaction between propagation method and rootstock cultivar was found.

The average monthly live root length during the first year after planting was significantly influenced by month (Fig. 1). Live root lengths increased continuously after planting until the summer. The largest net increase in live root length was observed between June and July, after which root length increased more slowly until December. Increase in live root length varied based on the propagation method, but there was a significant interaction with rootstock cultivar. The largest root growth was found for 'US-1516' propagated by tissue culture and the smallest for 'US-802' propagated by cuttings, followed by 'US-942' propagated by cuttings (data not shown).

Tree biomass distribution. The whole tree dry weight, and dry weights of leaves, scion (minus leaves), and rootstock (all tissue below the graft union) at the end of the study were not significantly influenced by the method of propagation, but significant differences were found among rootstock cultivars for all variables except rootstock weight (Table 4). The total tree dry weight was largest (3.6 kg) in trees on 'US-942' and smallest (1.9 kg) in trees on 'US-897', but separation of means was not statistically significant. Dry weight of leaves and scion were significantly higher (1.7 kg and 0.89 kg) in trees on 'US-942', and lower (0.8 kg and 0.53 kg) in trees on 'US-897'. No significant interactions between propagation methods and rootstock cultivars were observed, but block was a significant factor for all the variables.

The relative biomass distribution of leaves, scion, and rootstock, the rootstock to scion dry weight ratio, the total leaf area, and the SLA were not influenced by the rootstock-propagation method (Table 5). In contrast, the rootstock cultivar had a significant influence on most of the parameters measured. The leaf mass fraction was highest (47%) in trees on 'US-942' and lowest (39%) in trees on 'US-802'. The rootstock mass fraction was highest (36%) for 'US-802' and lowest (28%) for 'US-942'. The scion mass fraction was not significantly affected by the rootstock cultivar. The rootstock to scion dry

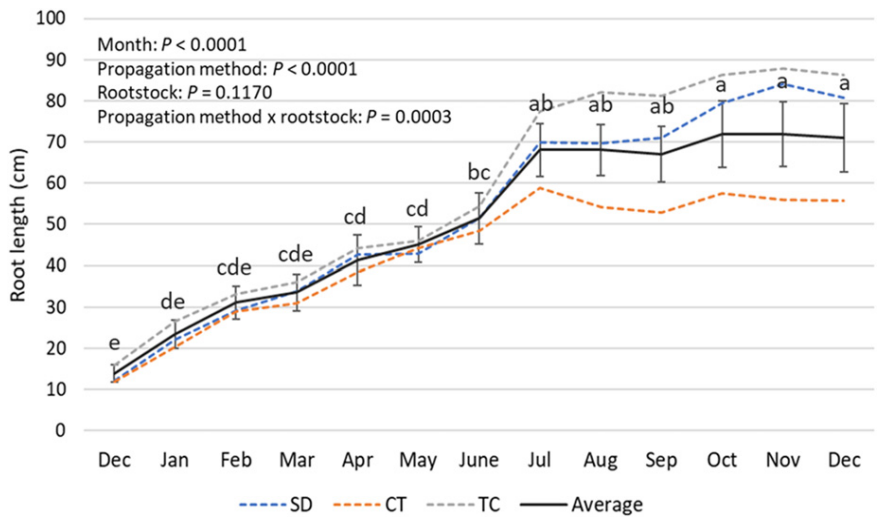

Fig. 1. Monthly average live root length of grafted 'Valencia' trees. Different letters indicate significant differences of average root lengths between months according to Tukey's honestly significant difference test. Live root length of rootstocks propagated by seed (SD), cuttings (CT), and tissue culture (TC) is indicated by dotted lines.

Table 3. Total root growth (length), live root length, and percentage of dead roots of different rootstocks propagated by seed (SD), cuttings (CT), and tissue culture (TC) and grafted with 'Valencia' scion from Dec. 2017 and Dec. 2018.

Factor	Total root length (m)	Live root length (m)	Dead roots (%)
Propagation method			
SD	1.7	0.81	47
CT	1.3	0.56	58
TC	1.5	0.86	46
P value	0.1688	0.1167	0.1663
Rootstock cultivar			
Swingle	1.4	0.66	56 ab
US-1516	1.7	1.13	30 b
US-802	1.5	0.74	40 ab
US-812	1.4	0.69	55 ab
US-897	1.7	0.75	55 ab
US-942	1.3	0.49	65 a
P value	0.6585	0.1242	0.0329*
Propagation method × rootstock			
P value	0.6024	0.9999	0.4185
Block			
P value	0.0009***	0.9999	0.6251
Block × rootstock			
P value	0.9494	0.9999	0.8676

Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. *, **, *** denote *P* values significant at 5%, 1%, and less than 0.1% level of significance.

Table 4. Dry weights of 2-year-old 'Valencia' trees on different rootstocks propagated by seed (SD), cuttings (CT), and tissue culture (TC).

Factors	Total tree wt (kg)	Leaf wt (kg)	Scion[z] wt (kg)	Rootstock[y] wt (kg)
Propagation method				
SD	3.0	1.3	0.75	0.93
CT	2.7	1.2	0.66	0.87
TC	2.6	1.2	0.64	0.76
P value	0.3097	0.4667	0.2969	0.1299
Rootstock				
Swingle	2.5 a	1.1 ab	0.61 abc	0.79
US-1516	2.2 a	1.0 ab	0.56 bc	0.67
US-802	2. 9 a	1.2 ab	0.68 abc	1.06
US-812	3.5 a	1.6 ab	0.84 ab	1.05
US-897	1.9 a	0.8 b	0.53 c	0.56
US-942	3.6 a	1.7 a	0.89 a	0.98
P value	0.0257*	0.0176*	0.0022**	0.0708
Propagation method × rootstock				
P value	0.5546	0.5980	0.7585	0.3136
Block				
P value	0.0134*	0.0126*	0.0068**	0.0455*
Block × rootstock				
P value	0.3091	0.4683	0.9999	0.0256*

[z]Minus leaves.
[y]All tissue below the graft union.
Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. *, ** denote *P* values significant at 5% and 1% level of significance.

Table 5. Leaf, scion, and rootstock mass distribution and other traits of 'Valencia' trees grafted on different rootstocks propagated by seed (SD), cutting (CT), and tissue culture (TC).

Factors	Ldw fraction (%)	Sdw fraction (%)	Rdw fraction (%)	Rdw/Sdw	Total leaf area (m^2)	SLA (cm^2/g)
Propagation method						
SD	44	26	31	0.46	9.1	67
CT	42	25	33	0.52	8.2	70
TC	45	26	30	0.43	8.3	70
P value	0.2637	0.7911	0.1114	0.0659	0.6397	0.3088
Rootstock						
Swingle	44 ab	25	32 ab	0.47 ab	6.9 ab	64
US-1516	43 ab	26	32 ab	0.49 ab	6.8 ab	71
US-802	39 b	24	36 a	0.61 a	8.5 ab	72
US-812	45 ab	25	30 ab	0.43 ab	11.4 a	71
US-897	44 ab	27	30 ab	0.42 ab	5.3 b	64
US-942	47 a	26	28 b	0.39 b	12.2 a	73
P value	0.0426*	0.2801	0.0422*	0.0292*	0.0081**	0.086
Propagation method × rootstock						
P value	0.4457	0.7921	0.2126	0.1267	0.3989	0.8979
Block						
P value	0.7321	0.0551	0.2577	0.2168	0.0058**	0.0215*
Block × rootstock						
P value	0.9999	0.9999	0.4994	0.7320	0.4424	0.0904

Ldw, Sdw, and Rdw = leaf, scion (minus leaves), and rootstock (all tissue below the graft union) dry weight. SLA = specific leaf area. Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. *, ** denote *P* values significant at 5% and 1% level of significance.

weight ratio varied significantly among trees on different rootstocks, with trees on 'US-802' having the highest (0.61) and trees on 'US-942' having the lowest (0.39) ratio.

The total leaf area was also significantly influenced by the rootstock cultivar. Trees on 'US-942' and 'US-812' had the largest (12.2 m^2 and 11.4 m^2) and trees on 'US-897' had the lowest (5.3 m^2) area. The SLA was not significantly affected by rootstock cultivar.

No significant interaction was observed between propagation method and rootstock cultivar for all parameters, but block was a significant factor for leaf area and SLA.

Root architecture and biomass distribution. The root system depth was 32 to 33 cm and not significantly different among rootstock-propagation types (Table 6); no dominant taproot was found for any of the trees (Fig. 2). The lateral root length varied significantly among propagation methods and was significantly larger in seed-propagated rootstocks (100 cm) than in tissue culture–propagated rootstocks (88 cm). No significant differences among propagation methods were found for the number of lateral roots and the lateral root diameter.

None of the root architecture traits varied significantly among rootstocks except the lateral root diameter, which was largest (7.3 mm and 7.4 mm) for 'US-942' and 'US-802', and smallest (5.2 mm) for 'Swingle'. There was no interaction between propagation method and rootstock for any of the root architecture parameters, but block was a significant factor for lateral root diameter.

The lateral root weight, lateral root mass fraction, fibrous root weight, fibrous root mass fraction, and SRL were not significantly influenced by the propagation method (Table 7). In contrast, significant differences were found among rootstocks for the lateral root weight and the lateral root mass fraction. The total lateral root weight ranged from 133 g for 'Swingle' to 326 g for 'US-942', but separation of means was not significant. The lateral root mass fraction was lowest for 'Swingle' and 'US-1516' (17% and 22%), and highest (33%) for US-942. There was no significant interaction between propagation method and rootstock cultivar.

Discussion

With the increasing demand for some of the best-performing rootstocks in an HLB-endemic environment, citrus nurseries have experienced a shortage of seeds, necessitating the use of alternative methods for propagation. Until now, there has been very little

Table 6. Root structural traits of different rootstocks propagated by seed (SD), cutting (CT) and tissue culture (TC) and grafted with 'Valencia' scion.

Factors	Root system depth (cm)	No. of lateral roots	Lateral root diam (mm)	Lateral root length (cm)
Propagation method				
SD	32	29	6.8	100 a
CT	32	34	6.0	97 ab
TC	33	29	6.3	88 b
P value	0.8498	0.0998	0.0654	0.0167*
Rootstock cultivar				
Swingle	33	37	5.2 c	94
US-1516	33	31	5.6 bc	79
US-802	33	26	7.4 a	94
US-812	35	30	6.9 ab	111
US-897	31	27	5.7 bc	96
US-942	30	33	7.3 a	95
P value	0.7816	0.2095	0.0005***	0.0835
Propagation method × rootstock cultivar				
P value	0.6759	0.2331	0.1912	0.1601
Block				
P value	0.6468	0.5811	0.0033**	0.4951
Block × rootstock cultivar				
P value	0.0392*	0.5130	0.7280	0.0114*

Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. *, **, *** denote *P* values significant at 5%, 1%, and less than 0.1% level of significance.

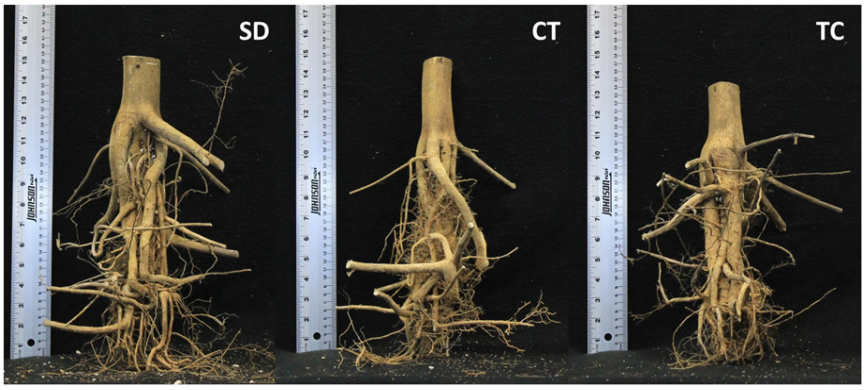

Fig. 2. Root crowns of seed- (SD), cuttings- (CT), and tissue culture– (TC) propagated rootstocks. The rootstock cultivar shown is US-802.

information about the relative influence of seed, tissue culture, and cutting propagation on the performance of citrus rootstocks in the field. This study presents novel information on the root architectures of cuttings and tissue culture–propagated rootstocks, and the influence on the grafted tree growth in comparison with seed-propagated rootstocks.

After 2 years of field growth, all trees were infected with CLas regardless of the rootstock-propagation method and the rootstock cultivar. Most of the aboveground horticultural traits were not influenced by the rootstock-propagation method, suggesting the suitability of cuttings and tissue culture–propagated rootstock for commercial citrus production. Among the few traits affected by the propagation method were the rootstock trunk diameter and, consequently, the scion to rootstock trunk diameter ratio, which was highest (closest to 1) for graft combination with cutting propagated rootstocks. The same was previously observed on 1-year-old field-grown 'Valencia' trees (Albrecht et al., 2020). The scion to rootstock trunk diameter ratio defines the smoothness of the graft union, which is often considered an indicator of the compatibility of the grafting partners (de Carvalho et al., 2018; Webber, 1948); however, differences in trunk diameters are also related to different vigors of the grafting partners (Bowman and Joubert, 2020). The lower rootstock trunk diameter and higher scion to rootstock trunk diameter ratio in graft combinations with cutting propagated rootstocks was likely because of a greater allocation of resources to the adventitious roots, which are most numerous in cuttings during the early stages of growth (Albrecht et al., 2017a, 2020).

In contrast to the rootstock-propagation method, many of the aboveground horticultural attributes varied among rootstock cultivars. This was not surprising, as these rootstocks have different parentages and characteristics (Bowman and Joubert, 2020) and are known to perform differently in terms of vigor and yield (Albrecht et al., 2020; Bowman et al., 2016a, 2016b). In the present study, trees on 'US-942' were found superior for most horticultural attributes, despite evidence for 'US-942' providing only medium vigor (Bowman et al., 2016b). Greenhouse studies have previously identified this rootstock as HLB tolerant (Albrecht and Bowman, 2012), which could be one reason for its superior performance under the HLB-endemic conditions of this study. The smallest trees were produced by 'US-897', which was expected, as this rootstock is known for its tree size–limiting effect (Bowman, 2007; Bowman et al., 2016a).

The leaf macro and micronutrient analyses conducted in 2018 and 2019 found no difference among propagation methods except for calcium, which was lowest in trees on tissue culture–propagated rootstocks in 2019. Based on the current guidelines for citrus (Kadyampakeni and Morgan, 2020), all nutrients were found in adequate concentrations. HLB is known to cause several nutrient deficiencies in affected trees (Pustika et al., 2008; Spann and Schumann, 2009), and nutrient management is an important component of mitigating HLB-induced tree decline (Morgan et al., 2016; Stansly et al., 2014). The lack of nutrient deficiencies observed in our study despite all trees testing positive for CLas was likely because of the proper nutrient management. In addition, the disease was still in a moderate stage of progression based on the results of the canopy health and disease symptom ratings.

The rootstock-propagation method did not influence the leaf, stem, and root mass fractions of trees, which is different from our previous observations on nursery-grown rootstock liners and grafted field-ready plants (Albrecht et al., 2017a, 2020). This suggests that initial differences in the biomass distribution associated with the propagation method diminish during the early years of growth in the field. The same was observed for 9-month-old *Eucalyptus* plants where some initial differences in architecture and morphology among tissue culture– and seed-propagated plants disappeared as the trees matured (Bell et al., 1993).

In contrast to the propagation method, tree biomass distributions were influenced by the rootstock cultivar. The leaf mass fraction and the total leaf area were largest for trees grafted on 'US-942'. The effect of 'US-942'

Table 7. Lateral and fibrous root masses, root mass fractions, and specific root length (SRL) of different rootstocks propagated by seed (SD), cutting (CT), and tissue culture (TC) and grafted with 'Valencia' scion.

Factors	Total lateral root wt (g)	Lateral roots (%)	Total fibrous root wt (g)	Fibrous roots (%)	SRL (m/g)
Propagation method					
SD	246	25	91	10	16
CT	222	23	99	11	17
TC	199	25	78	11	16
P value	0.3869	0.7087	0.2448	0.3054	0.8566
Rootstock cultivar					
SWG	133 a	17 b	85	11	14
US-1516	146 a	22 b	71	11	14
US-802	290 a	24 ab	96	9	16
US-812	285 a	25 ab	128	12	17
US-897	154 a	25 ab	53	10	20
US-942	326 a	33 a	102	10	16
P value	0.0215*	0.0002***	0.2012	0.7236	0.1138
Propagation method × rootstock cultivar					
P value	0.3517	0.5577	0.9413	0.5332	0.9412
Block					
P value	0.0685	0.4758	0.1896	0.9999	0.9461
Block × rootstock cultivar					
P value	0.1798	0.9999	0.0240*	0.3047	0.5659

Root mass fractions are expressed in relation to the total below graft union weight. Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. *, *** denote *P* values significant at 5% and less than 0.1% level of significance.

on leaf mass and area might be related to its tolerance to HLB (Albrecht and Bowman, 2012) and its general good adaptability to a wide range of stresses (Bowman and Joubert, 2020). The root mass fractions were largest for trees grafted on 'US-802', which is known for its vigor-inducing effect on the scion (Bowman et al., 2016a). Similar results were found in our previous study on field-ready grafted trees (Albrecht et al., 2020). Trees on 'US-802' also had the highest root to shoot mass ratio of all trees. This strong anchorage is essential, as trees on this rootstock can reach a height of more than 6 m (Bowman and Joubert, 2020) and require a well-developed root system for support.

It is commonly thought that having a long and strong taproot system is important for resisting wind-induced uprooting. Interestingly, no taproot was found in any of the excavated trees, and roots were shallow, occupying only the upper 36 cm of the soil. This contrasts with older studies on citrus, which described taproot systems penetrating 3 to 4 feet (90–120 cm) deep in the soil (Savage et al., 1945). Unlike those past studies, which were conducted on the sandy well-drained soils in central Florida, our study was conducted in southwest Florida where soils are poorly drained, preventing the formation of deeper roots (Freeland, 2016; Mylavarapu et al., 2016). Another contributing factor may be the switch from field to container production of citrus nursery trees, which began in Florida in ≈1977 (Zekri, 1999). Growth of citrus nursery rootstocks in containers generally limits the formation of a deep taproot before field planting, although this is affected by the particular container depth. A shallow soil penetration of roots was also found for 3-year-old citrus trees in Brazil, which had roots concentrated in the upper 40 cm of the soil (Meneses et al., 2020). Limited taproot growth was also observed in *Eucalyptus* trees, and it was suggested that anchorage is determined by the lateral roots (McComb et al., 1997). In fact, Dobson and Moffat (1995) noted that taproots are rarely found in mature trees and that horizontally growing (lateral) roots form at an early stage to provide the main structural support.

Resistance to wind-induced uprooting of citrus trees grown under the present production system in Florida is mainly determined by the root distribution and anchorage in the upper areas of the soil, which our results suggest is influenced more by the rootstock cultivar than the method of propagation. However, in field-grown *Eucalyptus* trees, a higher vertical uprooting resistance was observed in seed-propagated than micropropagated plants, which had a larger number of lateral roots (Mokotedi et al., 2010). In contrast, our study observed no difference in the number of lateral roots attributed to the propagation method. This is different from our previous studies, which found a larger number of lateral roots in cuttings during the nursery stage (Albrecht et al., 2017a, 2020). Therefore, considering the root depth and root distribution near the soil surface, the cuttings- and tissue culture–propagated rootstocks do not appear to be more vulnerable to wind-induced uprooting than seed-propagated rootstocks when grown under the conditions of this study. Whether the smaller rootstock trunk diameter of cuttings and the shorter lateral roots of tissue culture–propagated rootstocks will result in a higher vulnerability to windthrow is being investigated in an ongoing study.

The fine roots are the parts of the root system that is responsible for absorption of water and nutrients from the soil. HLB-affected trees have a reduced root biomass, and fibrous root loss was suggested to commence during the early presymptomatic disease stage (Graham et al., 2013; Johnson et al., 2013; Kumar and Kiran, 2018). It is therefore important to understand the dynamics of fibrous root production under HLB-endemic conditions. We found no influence of rootstock-propagation method on fibrous root mass production and SRL. Similar results were found in our previous study on field-ready nursery-grown citrus trees (Albrecht et al., 2020). In contrast, a study conducted by Castle (1977) with 9-year-old sweet orange trees on 'Milam' rootstock reported a larger feeder root weight in the upper zone of the soil for trees on cuttings than on seedlings and suggested a residual influence from the manner of root system development in young cuttings as a probable reason. In that study, trees were grown in the well-drained sandy soils of the Central Florida Ridge and under HLB-free conditions, which is different from our study. The field production of nursery trees at that time, as mentioned previously, may also have contributed to the residual influence of propagation method on the root system of 'Milam' rootstock.

Minirhizotron analysis showed a continuous pattern of root growth during the first months after planting followed by a higher rate of growth during the summer and little growth during the winter. A similar growth pattern was reported for 16-month-old 'Valencia' orange trees by Bevington and Castle (1985), who found continuous root growth from February to November with an increased intensity during the summer when soil temperatures were above 27°C. Citrus root growth is also known to follow shoot growth flush, especially after the main flushing period in spring (Bevington and Castle, 1985; Hall and Albrigo, 2007). Monthly net root growth was affected by the combination of rootstock and propagation method, suggesting that not all rootstock cultivars may be suited equally for the different propagation methods.

The total root growth measured by minirhizotron analysis during the first year after planting was unaffected by the propagation method or the rootstock cultivar. However, the percentage of dead roots varied among

rootstocks suggesting different capacities for root regeneration, which may influence tree tolerance to soil-borne diseases and to HLB.

Conclusion

Tissue culture and cutting propagation of rootstocks did not impair grafted tree performance compared with seed propagation during the first 2 years of growth in an HLB-endemic environment. Under the growing conditions of this field study, root structural traits were similar among differently propagated rootstocks and a deep taproot system was never observed. In contrast to the propagation method, most above- and below-ground tree traits were significantly influenced by the rootstock cultivar. This suggests a greater influence of the rootstock cultivar than the rootstock-propagation method on field tree performance. Longer-term investigation including the fruit production years will determine whether there is any difference in the economic potential for the use of seed-, cuttings-, and tissue culture–propagated rootstocks in a commercial production environment.

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Enhancing Strawberry Productivity Using Drip Irrigation, Plastic Mulch, and New Cultivars in Bangladesh

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Strawberry (*Fragaria ×ananassa*) is a relatively new crop in Bangladesh that has excellent potential since it can be grown during the cool season when less fruit is available. However, strawberry productivity is low due to the underutilization of improved production techniques. To address production constraints identified by Bangladeshi farmers, a study was conducted at Rajshahi University to compare the use of drip irrigation, plastic mulch and University of Florida (UF) cultivars with the current farmer practice in Bangladesh during the 2018–2019 season. The field trial was conducted using a split-plot design in which main plot treatments were either System 1 (drip irrigation with black plastic mulch) or System 2 (furrow irrigation with straw mulch) were arranged in a block design with restricted randomization and replicated four times. In the subplots, the UF strawberry cultivars 'Florida Radiance' and Sweet Sensation® 'Florida 127' were compared with Bangladeshi cultivars 'RU-2' and 'RU-3'. System 1 resulted in lower water use [655,578 gallons/acre (gal/acre)] than System 2 (994,381 gal/acre). System 1 plants had greater survival, vigor, and vegetative growth than System 2 plants. Flowering occurred earlier in System 1 than in System 2. Total marketable yield was generally higher with System 1 (697.5 g/plant) than with System 2 (450.6 g/plant). 'Florida 127' had the highest total marketable yield and produced fruits with higher total soluble solids than the other cultivars throughout the season. Therefore, a combination of drip irrigation with black plastic mulch and the 'Florida 127' strawberry cultivar appears to be the best option for enhancing strawberry productivity in Bangladesh.

As a relatively new crop in Bangladesh, knowledge about strawberry within the public and academic spheres is limited. However, strawberry production has been increasing during the cool season from November to April when many fruits commonly grown in Bangladesh are not available. Expansion of strawberry production in Bangladesh will increase fruit availability during this period of fruit scarcity (Ahmad and Uddin, 2012). Additionally, because strawberry production may allow the farmers of Bangladesh to earn lucrative profits from a small land area, their interest in the crop has been increasing. A farmer could easily earn $14,826 to $18,533 (US) from a strawberry crop on one hectare of land during the winter season (BSS, 2015). Including strawberry in existing crop rotations of rice and vegetables and between tree rows of young orchards may enhance the economic productivity of existing cropping systems.

Strawberry is now grown in a few regions of Bangladesh, with the greatest concentration in the northern region, which has the most favorable growing conditions. The climate in Bangladesh is sub-tropical with hot summers (May–August) and mild winters (December-February) (Rahman et al., 2015). Initially, only a few farmers were involved in strawberry cultivation, but the number of strawberry farmers has gradually increased as more farmers recognize that the crop can be commercially viable. However, Bangladesh has only a few locally developed strawberry cultivars namely BADC Strawberry, BARI Strawberry-1, 'RU-1', 'RU-2', and 'RU-3' (Chowhan et al., 2016; Rahman, 2009). Farmers usually use the indigenous strawberry cultivars; however, some research stations have evaluated imported cultivars such as 'Strawberry Festival', 'Florida Radiance', 'Sweet Charlie', 'Camarosa', and 'FL 05-107' (Chowhan et al., 2016; Rahman et al., 2015).

Selecting adapted cultivars is considered by Asrey and Singh (2004) to be the most important factor for fruitful strawberry production. However, the performance of strawberry cultivars also depends on transplant quality (Johnson et al., 2005; Le

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Miere et al., 1998; Perez de Camacaro et al., 2004; Shokaeva, 2004). As a relatively new crop in Bangladesh, research emphasis has been placed on comparing strawberry cultivar yield, yield contributing characteristics, and fruit quality (Rahman et al., 2015). In previous work, Biswas et al. (2009) focused on using somaclonal variation to develop new cultivars. Although BARI Strawberry-1, a released of the Bangladesh Agricultural Research Institute, had the higher total soluble solids than 'Camarosa' and 'Strawberry Festival' it was considered to be unable to meet the increasing demand for strawberries in Bangladesh due to low yields (Chowhan et al., 2016). Genotypes FA 005, FA 006, and FA 007 from local and exotic sources performed well based on physico-chemical characteristics among thirteen genotypes tested in subtropical climates (Rahman et al., 2015).

In addition to better cultivars, technology such as plasticulture and drip irrigation may also have potential for improving strawberry productivity. Plasticulture systems have been shown to provide better soil moisture conservation, weed control, fertigation application efficiency, fruit size, and early yield than a conventional matted row system (O'Dell and Williams, 2000). In the Sylhet district of Bangladesh, micro-drip irrigation with plasticulture enhanced plant growth and development, reduced chemical use and production cost, and improved fruit quality and yield of tissue culture plantlets of strawberry compared to the conventional practice with no mulch and manual irrigation (Zobayer et al., 2011). While black plastic mulch and straw mulch was compared for strawberry production by Rannu et al. (2018), their study evaluated a single germplasm line (FA-007) and did not evaluate drip irrigation.

Farmers in Bangladesh are commonly using the 'RU-2' and 'RU-3' strawberry cultivars, which are characterized by good canopy size, large fruits, uniform color, anthracnose resistance and relatively higher yield among the Bangladeshi cultivars (Rahman, 2009). Cultivars developed for use in Florida may prove to be well-adapted to the subtropical conditions in Bangladesh and may offer improved quality and productivity compared to local cultivars. 'Florida Radiance' (marketed as 'Florida Fortuna' outside of the USA) was released in 2008 by the University of Florida (UF) (Chandler et al. 2009) and is adapted to regions where winter and early spring production is practiced. While having a number of desirable characteristics that make it well suited to the winter plasticulture production system, fruit firmness is just acceptable and disease susceptibility requires careful management to limit plant and fruit loss due to pathogens (Whitaker et al., 2016). A more recently released UF cultivar, Sweet Sensation® 'Florida 127' is also highly adapted to the winter plasticulture growing system, and produces a plant that is compact, robust and upright with long pedicels that facilitate easy harvesting (Whitaker et al., 2017). The fruits are uniform and large with prolonged shelf life and high Brix content. The plants are resistant to anthracnose but susceptible to botrytis. Low strawberry productivity in Bangladesh is likely due to the underutilization of improved production techniques. To address production constraints identified by Bangladeshi farmers as a means of improving strawberry productivity and quality, a study was undertaken to compare the use of drip irrigation, plastic mulch and new cultivars with the current farmer practice in Bangladesh.

Although common in many other areas with subtropical strawberry production, the annual hill (raised bed) system with plastic mulch and drip irrigation is not well understood by Bangladeshi farmers. As a result, there is a need for relevant research and extension of these methods to strawberry farmers. Therefore, the objective of this study was to evaluate drip irrigation, plastic mulch, and University of Florida cultivars for improved strawberry productivity in Bangladesh.

Materials and Methods

A field trial was conducted at Rajshahi University (RU) (lat. 24°36'N, long. 88°64'E), Bangladesh, during the 2018–19 growing season. The soil texture of the RU experimental site is classified as clay loam with 2.5% organic matter and pH of 6.9 (Islam et al., 2017). The experimental land was prepared by tillage to an approximate depth of 15 cm (Power tiller, SIFANG-121, Sifang, China). The trial was conducted using a split-plot design in which main plot treatments were either System 1 (drip irrigation with black plastic mulch) or System 2 (furrow irrigation with straw mulch) were arranged in a block design with restricted randomization and replicated 4 times. The subplot treatments were strawberry cultivars: 'RU-2', 'RU-3', 'Florida Radiance,' and Sweet Sensation® 'Florida 127'. The plug transplants of 'RU-2' and 'RU-3' were obtained from Akafuji Agro-technologies farm (Rajshahi, Bangladesh) and 'Florida Radiance,' and 'Florida 127' plug transplants were obtained from an Ekland Marketing Company-affiliated nursery in India (Zopar Exports Pvt. Ltd., Aizawl, Mizoram, India). Strawberry cultivars were randomly assigned to the subplots. Beds were prepared manually on 1.2-m centers with approximate bed heights and widths of 18 cm and 71 cm for System 1 and 10 cm and 71 cm for System 2, respectively.

For System 1 black plastic mulch (0.09-mm thickness; BPS Industries Corp., Beijing, China) was laid manually on the surface of raised beds prior to transplanting, and a single drip tape line (7.5 L/30 m, 0.03-mm thickness, 140-mm diameter, 30.5-cm emitter spacing, Advanced Plastic Technology, Barletta, Italy) was placed along the center of the bed during the laying of the mulch film. System 2 utilized locally sourced straw mulch. Colored spray paint (for plastic mulch plots) and wooden stakes (for straw mulch plots) were used to divide the beds into plots 2.29 m in length. At RU, 14 strawberry plug transplants per plot were planted in two offset rows per bed with a between-row spacing of 30.5 cm and a 38-cm within-row spacing on 26 Nov. 2018. Planting holes were punched through the plastic mulch using metal stakes to facilitate transplanting.

Crop Management. Drip irrigation was applied in System 1 twice a day for 55 minutes per application until December, and 70 minutes per application from January to March to maintain optimal soil moisture content for strawberry growth and yield (Sanchez, 2018). Water was supplied by gravity feed from an elevated water tank (500 L, Gazi Tank, Dhaka, Bangladesh), which was filled by an electric pump. In System 2, irrigation was applied at 15-day intervals after transplant establishment. During establishment, transplants were irrigated with a watering can (1.5-L Plastic Can, Fixit.Com.BD, Dhaka, Bangladesh). Soil moisture was monitored at 7-d interval in each system using a tensiometer (Irrometer, Model-P, Shelburne, VT 05482, Part number-P-2001, portable soil water tension detector; Mega Depot, LLC, Hinesburg, VT).

Diammonium phosphate (68 kg/ha) and muriate of potash (17 kg/ha) were applied as basal fertilizers during land preparation for System 2. A mixture of urea, muriate of potash, calcium nitrate, magnesium nitrate, zinc sulfate, and borax (6:8:2:0.4:0.02:0.02) at 5.9 kg/week/ha was applied throughout the season via the drip tape in System 1 and as manual spray applications for System 2. All fertilizers were purchased from the local Bangladesh market.

Data collection. Data were collected from the inner the plants within each row to avoid border effects. Plant vigor was assessed using a scale of 0 to 10 with 0 being dead plants and 10 being the most vigorous plants, a slightly modification of the method used by Monfort et al. (2007) (0–10 rating used instead 1–10). Strawberry transplant survival was assessed at 2 and 4 weeks after transplanting and expressed as a percentage. Days to first flowering was assessed as the number of days from planting to the first flower opening in each plot. Numbers of runners and leaves were assessed from six randomly selected plants per plot. Crown size was measured on four randomly selected plants using digital slide calipers. A ruler was used to measure the width and length of the canopies of four randomly selected plants and canopy size was calculated as an area by multiplying the two measurements.

Strawberries were harvested by hand early in the day while air temperatures were cool, every 3–4 d. The fruit was harvested at commercial maturity after > 80% of the fruit surface turned a uniform red color. Immediately after harvest, strawberries were sorted into marketable and unmarketable (cull) fruits. The number and fresh weight of marketable and cull fruits were determined from 10 sample plants per plot. The fruit yields from January through March are considered the total yield. Total soluble solids (TSS) were measured as degrees Brix once per month (Digital/Brix/RI-Check Refractometer, Reichert Technologies, Inc. Japan).

Statistical analysis. Data were analyzed using the GLIMMIX procedure of SAS Version 9.4 (SAS Institute Inc, Cary, NC). The main effects of irrigation system and cultivar and their interaction were considered fixed. Least squares mean separation was accomplished using the Tukey-Kramer adjustment. The level of significance used was 5%. Repeated measures analysis was used for variables for which the effect of the treatments on the same experimental unit at several different times.

Results

Soil moisture. More uniform soil moisture was observed with System 1 than with System 2 throughout the season (Fig. 1). On 22 Feb., soil moisture was very high in both systems due to heavy rainfall. Irrigation water use was lower with System 1 than System 2, which was expected (Table 1). This was because the drip irrigation could be controlled more precisely than the furrow irrigation. System 1 (655,578 gal/acre) provided more efficient water use with 338,803 gal/acre less water used than System 2 (994,381 gal/acre).

Table 1. Comparisons of irrigation water use in drip irrigation and furrow irrigation systems

System (S)	Duration	GPA[z]
S1 = Drip irrigation + plastic mulch	26 Nov.–29 Mar.	655,578
S2 =Furrow irrigation + straw mulch	26 Nov.–29 Mar.	994,381
Difference	–	338,803

[z]GPA = gallons per acre (gal/acre).

Plant survival, vigor, crown diameter and days to first flower. Significantly higher plant survival was obtained with System 1 than System 2 (Table 2). Transplant mortality was higher with 'Florida Radiance' compared to the other strawberry cultivars tested. A similar trend was found for plant vigor in both systems. In the case of vigor, 'Florida 127' performed better than 'Florida Radiance' and 'RU-2' but did not differ from 'RU-3'. A significant difference in days to first flower was obtained with the two systems. Earlier flowering occurred with System 1 than with System 2. However, there was no difference in days to flowering among the strawberry cultivars.

At 8 and 12 weeks after transplanting, there was a significant system by cultivar interaction for crown diameter and, therefore, the simple effects of system and cultivar were assessed for these sample dates (Table 2). However, the system by cultivar interaction was not significant at 4 and 16 WAT for crown diameter and the main effects of system and cultivar are presented for these dates. At all four sample dates crown diameter was greater for System 1 than System 2. When cultivars were compared, 'Florida 127' had larger crown diameter than 'Florida Radiance' and 'RU-2' with no statistical differences with 'RU-3' at 4 WAT. At 8 and 12 WAT no difference in crown diameter was apparent among the cultivars in System 1; however, in System 2 'Florida 127' had the largest crown diameter. 'Florida Radiance' had the smallest crown diameter at 8 WAT in the System 1 with no difference observed among 'Florida Radiance', 'RU-2', and 'RU-3' at 12 WAT. By 16 WAT. Averaged over system, 'Florida127' had the largest crown diameter of the four cultivars.

Leaf number, canopy size, and runner number. There was no interaction between system and cultivar for leaf number at 4 and 8 WAT but a significant system by cultivar interaction was found at 12 and 16 WAT (Table 3). Leaf number was higher with System 1 than System 2 at 4 and 8 WAT. 'Florida 127' produced more leaves than 'Florida Radiance' and 'RU-2' but leaf number was not statistically different from that of 'RU-3' at 4 WAT. Cul-

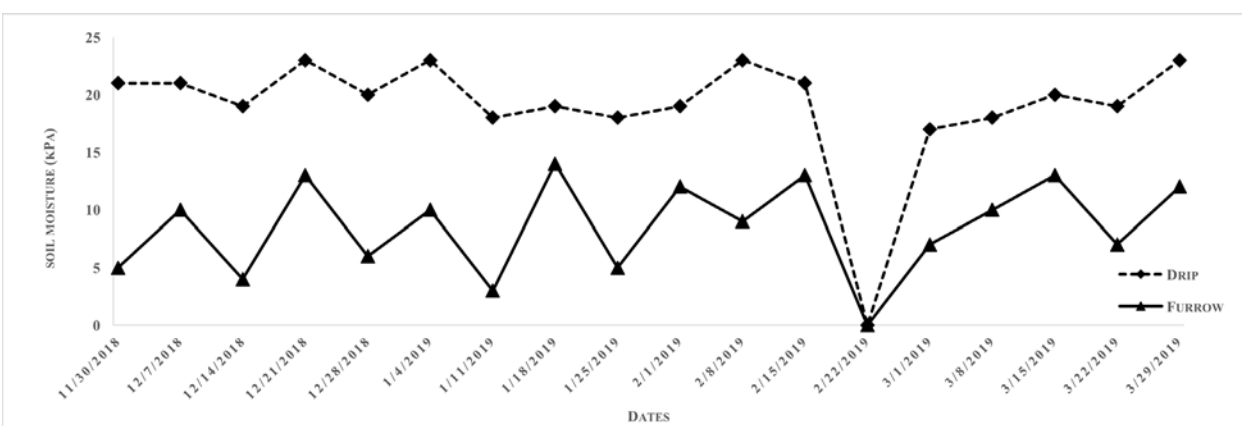

Fig. 1. Soil moisture (kPa) at a weekly interval in drip and furrow irrigation systems measured using tensiometers.

Table 2. Effect of production system and strawberry cultivar on plant survival, vigor, days to first flower, and crown diameter.

Treatments	Plant survival (%)		Plant vigor		Days to	Crown diameter (cm)					
	2 WAT[z]	4 WAT	2 WAT	4 WAT	first flower	4 WAT	8 WAT		12 WAT		16 WAT
System (S)											
S1[y]	91.5 a	87.5 a	6.3 a	6.9 a	36.9 b	0.85 a	–		–		3.5 a
S2[x]	73.7 b	71.0 b	3.9 b	4.6 b	40.9 a	0.70 b	–		–		2.6 b
Cultivar (C)							S1	S2	S1	S2	
Florida 127	83.9 a	79.5 a	6.0 a	6.8 a	38.3	0.80 a	1.6 a	1.3 d	2.8 a	2.0 c	3.2 a
Florida Radiance	71.4 b	67.9 b	4.3 b	5.3 b	39.1	0.75 b	1.4 c	1.2 d	2.3 b	1.9 c	2.9 b
RU-2	85.7 a	83.9 a	4.9 b	5.1 b	39.5	0.76 b	1.5 b	1.2 d	2.4 b	2.0 c	3.0 b
RU-3	89.3 a	85.7 a	5.1 ab	5.9 ab	38.9	0.77 ab	1.5 b	1.2 d	2.5 b	2.1 c	3.0 b
Significance											
System (S)	**	**	***	***	***	***	***		***		***
Cultivar (C)	***	***	***	**	NS	**	***		***		***
S × C	NS	NS	NS	NS	NS	NS	***		***		NS

[z]WAT = weeks after transplanting.
[y]S1 = drip irrigation with black plastic mulch.
[x]S2 = furrow irrigation with straw mulch.
Least squares means in columns followed by the same letters do not differ significantly according to the Tukey-Kramer test.
NS, **, *** indicate nonsignificant or significant at $P < 0.01$ and 0.001, respectively.

Table 3. Effect of production system and strawberry cultivar on leaf number and canopy size[z].

Treatments	Leaf number/plant						Canopy size (cm^2)			
	4 WAT[z]	8 WAT	12 WAT		16 WAT		4 WAT	8 WAT	12 WAT	16 WAT
System (S)										
S1[y]	5.6 a	10.6 a	–		–		67.6 a	158.3 a	237.8 a	330.4 a
S2[x]	4.5 b	7.9 b	–		–		40.5 b	85.3 b	163.2 b	232.3 b
Cultivars(C)			S1	S2	S1	S2				
Florida 127	5.6 a	9.6	23.0 a	14.3 c	36.5 a	23.5 c	55.9	132.7	217.8	299.7
Florida Radiance	4.5 c	8.8	18.3 b	14.0 c	31.8 b	21.5 c	49.8	111.1	188.3	268.6
RU-2	5.6 bc	9.1	20.3 ab	14.3 c	29.3 b	21.5 c	55.6	121.6	199.7	281.0
RU-3	5.5 ab	9.6	18.5 b	13.8 c	31.5 b	23.0 c	54.9	121.6	196.2	275.9
Significance										
System (S)	*	***	***		**		*	***	*	***
Cultivar (C)	**	NS	**		***		NS	NS	NS	NS
S × C	NS	NS	**		*		NS	NS	NS	NS

[z]WAT = weeks after transplanting.
[y]S1 = drip irrigation with black plastic mulch.
[x]S2 = furrow irrigation with straw mulch.
Least squares means followed by the same letters within columns do not differ significantly according to the Tukey-Kramer test.
NS, *, **, *** indicate nonsignificant or significant at $P < 0.05$, 0.01, and 0.001, respectively.

tivars did not differ in leaf number at 8 WAT. 'Florida 127' with System 1 and produced more leaves than 'Florida Radiance' and 'RU-3' at 12 WAT and had more leaves than all other cultivars at 16 WAT (Table 3). The canopy size of strawberry plants in System 1 was larger than in System 2 throughout the season. However, strawberry cultivars did not differ in canopy size at any of the sample dates. Runner production was very low and there was no significant effect of treatments at 4, 8, 12, and 16 WAT (Table 4).

Total soluble solids and fruit yield. For TSS, the system by cultivar interaction was not significant in January, but a significant interaction was observed in February and March. In January, System 1 produced sweeter fruits than System 2 (Table 4). 'Florida 127' resulted in sweeter fruits than other cultivars. In System 1 'Florida 127' produced sweeter fruits than the other cultivars in February and March. Only 'Florida 127' resulted in higher TSS in System 1 than System 2 in February and March. For the other three cultivars there was no difference in TSS between the systems. In general, 'Florida Radiance' had higher TSS than both 'RU-2' and 'RU-3'.

In the case of number of marketable fruit number and weight in January, a significant system by cultivar interaction was obtained (Table 5). In System 1, 'Florida 127' produced more fruits than 'Florida Radiance' and 'RU-2', but its marketable fruit number was not different from that of 'RU-3'. For fruit weight in January, in System 1 marketable fruit weight was highest with 'Florida 127'. In System 2 there was no cultivar difference in either fruit number or fruit weight in January.

There was no system by cultivar interaction for marketable fruit number and fruit weight in February and March and for season total marketable fruit number and fruit weight (Table 5). The System 1 produced more fruits than System 2. In February, 'Florida 127' produced more fruits and greater fruit weight than 'RU-3' with no difference when compared with 'Florida Radiance' and 'RU-2'. In March, 'Florida 127' produced more fruits

Table 4. Effect of production system and strawberry cultivar on runner number and total soluble solid.

Treatments	Runner number				Total soluble solids (°Brix)				
	4 WAT[z]	8 WAT	12 WAT	16 WAT	Jan.	Feb.		Mar.	
System (S)									
S1[y]	1.3	1.5	1.6	1.3	7.3 a	–		–	
S2[x]	1.2	1.4	1.6	1.4	7.1 b	–		–	
Cultivar (C)						S1	S2	S1	S2
Florida 127	1.0	1.6	1.5	1.5	8.1 a	8.9 a	8.2 b	9.2 a	8.6 b
Florida Radiance	1.1	1.3	1.4	1.1	7.3 b	7.6 c	7.7 c	8.0 b	8.1 b
RU-2	1.4	1.6	1.8	1.3	6.6 c	7.0 d	6.9 d	7.1 c	7.2 c
RU-3	1.4	1.4	1.8	1.4	6.8 c	7.1 d	6.9 d	7.2 c	7.1 c
Significance									
System (S)	NS	NS	NS	NS	*	**		NS	
Cultivar (C)	NS	NS	NS	NS	***	***		***	
S × C	NS	NS	NS	NS	NS	***		*	

[z]WAT = Weeks after transplanting

[y]S1 = Drip irrigation with black plastic mulch.

[x]S2 = Furrow irrigation with straw mulch.

Least squares means in columns followed by the same letters do not differ significantly according to the Tukey-Kramer test.

NS, *, **, *** indicate nonsignificant or significant at $P < 0.05$, 0.01, and 0.001, respectively.

and greater fruit weight than 'RU-2' and 'RU-3' with no differences when compared with 'Florida Radiance'. In the case of season total marketable fruit number and fruit weight, 'Florida 127' produced more yield than the other cultivars. 'Florida 127' had a larger fruit size (data not shown) and produced more fruits than other cultivars possibly explaining some of the cultivar differences in this study.

In contrast, no significant effect on number of unmarketable fruits was observed due to system and cultivar (Table 5). Also, unmarketable fruit weight was not significant throughout the

Table 5. Effect of production system and strawberry cultivar on yield of marketable and unmarketable (cull) fruits.

Treatments	Marketable fruit number/plant					Cull fruit number/plant			
	Jan.		Feb.	Mar.	Total	Jan.	Feb.	Mar.	Total
System (S)									
S1[z]	–		16.4 a	25.3 a	52.6 a	4.9	7.0	13.0	24.9
S2[y]	–		9.7 b	19.1 b	36.4 b	5.1	8.3	12.0	25.4
Cultivar (C)	S1	S2							
Florida 127	13.7 a	7.8 bc	14.6 a	24.6 a	50.0 a	5.1	7.6	12.0	24.7
Florida Radiance	10.0 bc	8.3 bc	13.0 ab	22.0 ab	44.1 b	4.9	8.0	12.8	25.7
RU-2	9.3 bc	7.3 c	12.8 ab	21.1 b	42.1 b	4.3	6.8	12.6	23.7
RU-3	11.0 ab	7.3 c	11.8 b	21.0 b	41.9 b	5.6	8.1	12.6	26.3
Significance									
System (S)	**		*	**	**	NS	NS	NS	NS
Cultivar (C)	*		*	*	**	NS	NS	NS	NS
S × C	**		NS	NS	NS	NS	NS	NS	NS
	Marketable fruit wt (g/plant)					Cull fruit wt (g/plant)			
Treatments	Jan.		Feb.	Mar.	Total	Jan.		Feb.	Mar.	Total
System (S)										
S1	–		229.3 a	320.5 a	697.5 a	–		44.8	83.4	158.9
S2	–		122.6 b	235.3 b	450.6 b	–		49.9	77.1	152.6
Cultivar (C)	S1	S2				S1	S2			
Florida 127	185.3 a	95.5 cd	200.3 a	310.3 a	650.9 a	39.8 a	21.8 ab	47.9	78.9	157.5
Florida Radiance	137.5 bc	98.8 bcd	177.5 ab	274.3 ab	569.9 b	31.5 ab	18.5 b	45.1	74.9	145.0
RU-2	126.0 bcd	90.8 d	164.6 b	264.4 b	537.4 b	23.5 ab	29.8 ab	42.8	86.9	156.3
RU-3	142.0 b	85.8 d	161.5 b	262.6 b	538.0 b	28.0 ab	32.8 ab	53.6	80.4	164.4
Significance										
System (S)	**		*	**	**	NS		NS	NS	NS
Cultivar (C)	**		**	*	***	NS		NS	NS	NS
S × C	**		NS	NS	NS	**		NS	NS	NS

[z]S1 = Drip irrigation with black plastic mulch.

[y]S2 = Furrow irrigation with straw mulch.

Least squares means in columns followed by the same letters do not differ significantly according to the Tukey-Kramer test.

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season except in January when the system by cultivar interaction was significant. This was due to the highest unmarketable fruit weight occurring with 'Florida 127' in System 1 and the lowest unmarketable fruit weight occurring with 'Florida Radiance' in System 2.

Discussion

The drip irrigation system allowed for the maintenance of soil moisture within the top 30–40 cm of soil where much of the strawberry root system occurs. Drip irrigation use also helps to avoid excessive water loss due to percolation and infiltration (Morillo et al., 2017). In India, popularity and adoption of drip irrigation has expanded due to better water use efficiency and ensured higher yield with various crops (Panigrahi et al., 2012). Water use efficiency was higher with a drip irrigation system than a furrow irrigation system in research findings reported by Hoppula and Salo (2007) and Kumar et al. (2012). This is due to the fact that drip irrigation supplies water directly to the root zone with less water loss by evaporation and less leaching of soil-applied fertilizers compared with surface irrigation (Sharma et al., 2005).

Drip irrigation maintained more uniform soil moisture with more consistent plant growth and development than furrow irrigation in this study. A similar response was observed by Zobayer et al. (2011) in Sylhet Bangladesh. They mentioned that plant growth and development of strawberry transplants propagated using tissue culture were better with the micro-drip irrigation and plasticulture system. A similar response was obtained in this study in which System 1 with black plastic mulch and drip irrigation resulted in better plant growth than System 2, a more traditional system with less frequent furrow irrigation and straw mulch. Drip irrigation gave higher yield than the furrow irrigation system. These results are in agreement with the findings of Fahad and Hagemann (1992), Kachwaya et al. (2016), Minami et al. (1982), and Yuan et al. (2004).

Ahmad et al. (2014) reported that groundwater use is unsustainable in parts of Bangladesh and that its use for irrigation is threatened in some areas due to falling water tables. Rajshahi, where strawberries are grown, was identified as an area with low water productivity. To address this constraint, changing to more sustainable agronomic practices along with improved technology and promoting its adoption were recommended by Ahmad et al. (2014). The cost of new technology such as plastic mulch, drip irrigation, and new cultivars is often a barrier to adoption by growers. However, government subsidies for agriculture may be available to offset the cost of new technology through programs such as the National Agricultural Technology Project, which are intended to increase agricultural productivity and farm income (World Bank, 2014).

Adnan et al. (2017) observed significant variation in strawberry plant growth due to variety and mulch effect. They found that black plastic mulch resulted in better plant growth and higher strawberry flower number per plant than water hyacinth mulch and straw mulch. An increase in strawberry flower number per plant due to use of black and silver mulch was also reported by Ali and Redwan (2008). A similar response was observed in this study where black plastic mulch with drip irrigation system resulted in earlier flowering compared to straw mulch with furrow irrigation. Black plastic mulches are generally used during the cool season when they can warm the soil, enhance growth, and contribute to early yields. Not only do organic mulches limit soil warming, but those with high carbon to nitrogen ratios can also affect nitrogen availability to the crop. Number of fruits per plant, fruit fresh weight and fruit number were highest with black plastic mulch when compared with water hyacinth mulch and straw mulch (Adnan et al., 2017), which is consistent with the results from this experiment. Black mulch facilitated the growth and development of strawberry plants in several other studies resulting in higher fruit yield than straw mulch (Baumann et al. 1995; Shiow et al., 1998; Vander Meulen et al. 2006).

Previous evaluations of strawberry germplasm in Bangladesh showed that strawberry traits such as uniformity, shape, color, and fruit yield varied with genotype (Rahman et al., 2013, Rahman et al., 2014, Rahman et al., 2015). Although strawberry yield potential can vary due to cultivar (Himelrick et al., 1993; Himelrick and Akridge, 1999), genotype alone may not account for the difference in productivity observed with the two Florida cultivars. 'Florida 127' resulted in the highest marketable fruit yield in this study, out yielding even 'Florida Radiance'. This was a curious result since in Florida, Whitaker et al. (2015) found no significant difference in the per plant yield of 'Florida 127' and 'Florida Radiance' over two consecutive seasons. It is possible that 'Florida 127' is better adapted to growing conditions in Rajshahi where the soil is a clay loam with a higher organic matter content than the sandy soil in Florida where the previous study was conducted. Better adaptability may also explain less mortality, greater vigor, and larger crown diameter by 16 WAT with 'Florida 127' than with 'Florida Radiance'. Further, the Rajshahi study was conducted without soil fumigant, fungicides, and pesticides. Thus, differential susceptibility of the two cultivars to biotic and abiotic conditions may account for the lower yield in 'Florida Radiance' in Rajshahi. In the current study, the highest TSS content was observed in 'Florida 127' among the four cultivars tested. This result is consistent with the performance of 'Florida 127' in Florida where soluble solids content and trained sensory panels ratings were higher in more assessments with 'Florida 127' than with 'Florida Radiance' (Whitaker et al., 2015).

In general, drip irrigation with black plastic mulch resulted in lower water use and better growth and yield of strawberry than furrow irrigation and straw mulch. 'Florida 127' resulted in higher marketable yields and higher total soluble solids content than 'Florida Radiance' and two Bangladeshi cultivars. Therefore, a combination of drip irrigation with black plastic mulch and the Florida 127 strawberry cultivar appears to be the best option for enhancing strawberry productivity in Bangladesh. The study will need to be repeated before recommendations can be made. Options for providing growers with legally sourced, high quality 'Florida 127' transplants will also need to be explored.

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Management of Diamondback Moth, *Plutella xylostella* (Lin.) (Lepidoptera: Plutellidae) In Cabbage using Reduced-risk Insecticides

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Keywords. cabbage, Diamondback moth, reduced-risk insecticide

Cabbage is a valuable crop in Florida as it produces 13% of the nation's fresh market crop. Diamondback moth (DBM) (*Plutella xylostella* L.) is the most destructive pest of cabbage and other cruciferous crops. Even though growers use sound chemical control strategies to manage this serious pest, they cannot truly control it and are also increasing the possibility of insecticide resistance. In the present study, we evaluated different reduced-risk insecticides to manage DBM larvae and pupae. The insecticides Xentari-Knack, Entrust, BioProtect, and *Bacillus thuringiensis*, subsp. *aizawai*, Strain ABTS-1857 with pyriproxyfen, and Spear in combination with BioProtect were significantly reduced both DBM larvae and pupae in cabbages than the other insecticides and untreated control. Information from this study may be helpful to local growers to use some reduced-risk insecticides to avoid the resistance problem and increase yield.

Florida produces 13% of US cabbage and ranks third nationally in the production of fresh market cabbage. Cabbage, *Brassica oleracea* var. *capitata* L. is the nineth most important vegetable crop in Florida (Florida Department of Agriculture and Consumer Services 2012). Florida growers plant cabbages annually on more than 17,000 acres (Hochmuth, 1988, Freeman et al. 2021). Insect pests are the main constraint to growing cabbage in Florida. Among these, the diamondback moth (DBM) (*Plutella xylostella* L.) is considered the most destructive pest of cabbage. The pest is from the southern Africa and is an invasive species to the United States (Talekar and Shelton 1993). The larval stages are the most destructive life stages of DBM. Crop damage is initiated by larval feeding and the presence of larvae contaminating produce (Shelton et al. 2000). The first instar larvae mine leaf tissue and the later instars consume leaf tissue from the underside of the leaves, giving them a window-like appearance (Philips et al. 2014). The high reproductive potential, the lack of natural enemies in nature and the ability to become resistant to a wide variety of chemicals have established DBM as the pest most successful at challenging modern pest management approaches. Diamondback moth is a widely distributed serious insect pest of Brassica and costs the world economy $4–5 billion (US) annually (Zaluchi et al. 2012). Historically, the DBM was managed by repeated insecticide applications which lead to the development of resistance (Furlong et al. 2013). In the North America, the most severe cases of DBM resistance has been reported from the southern US (Shelton and Wyman, 1993).

Insecticides with different mode of actions have been applied to manage DBM and increase crop yields. The increase in resistance of insect pests, killing natural enemies of insect pests, and environmental pollution are all harmful effects of the intensive use of chemical insecticides (Barriuso and Koskinen 1996; Liu et al., 1982). Diamondback moth has evolved resistance to at least 79 insecticides from a variety of insecticidal classes including carbamates, pyrethroids, organophosphates, Spinosad, abamectin, and *Bacillus thuringiensis*-based products (Liu et al., 1981; Kobayashi et al., 1993; Sun et al. 2012). Shelton and Wyman (1990) reported that the North American populations of DBM are > 100-fold resistant to pyrethroids and some carbamates and > 200-fold to *B. thuringiensis*. However, the level of insecticide resistance varies by region (Cordero and Kuhar 2007). Diamondback moth has a remarkable ability to rapidly develop resistance (Philips et al. 2014) after only a few generations. Therefore, extending the intervals between applications of the same insecticide can delay the development of resistance (Tabashnik 1986). However, growers are using chemical treatments as the principal tool to save their crop from DBM (Mazlan and Mumford.2005, Grzywacz et al. 2010).

Alternative management strategies need to be evaluated to reduce the harmful effects of chemical insecticides and insect resistance while developing a sound integrated management program. In the present study, we made some efforts to manage the diamondback moth using various reduced-risk insecticides. In different research experiments, we used Azadirachtin, *Bacillus thuringiensis*, subsp. *kurstaki*, strain ABTS-351, *Bacillus thuringiensis* subsp. *aizawai*, Strain ABTS-1857, pyriproxyfen, 3-(trihydroxysilyl)propyldimethyloctadecyl ammonium chloride, spinosad, and *Chromobacterium subtsugae* strain PRAA4-1^{T} to determine their efficacy in managing DBM larvae and pupae in cabbage.

Materials and Methods

First study. 'Gourmet' cabbage transplants were set in an experimental field in Mar. 2020 at the Tropical Research and Education Center, Homestead, FL. The soil type was Krome gravelly loam (loamy-skeletal, carbonatic hyperthermic lithic

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Udorthents), which consists of about 33% soil and 67% pebbles (> 2 mm). Beds were covered with polyethylene mulch Each plot consisted of two parallel raised beds 36 inches wide, 25 ft long, and separated by a 5 ft alley. Plants were spaced 18 inches apart within each row. Trifluralin (Treflan® 4EC) was incorporated into the soil of each bed 2 weeks before planting. Plants were fertilized by applying granular fertilizer (6 N–12 P–12 K) at 1345 kg/ha in a 10-cm-wide band on each side of the center of each raised bed, which was then incorporated before placement of plastic mulch. Liquid fertilizer (4 N–0 P–8 K) was also applied at 0.56 kg/ha N through a drip system at 2, 3, 4, and 5 weeks after planting. Plants were irrigated every day for one hour using two parallel lines of drip tube (T-systems, DripWorks Inc., Willits, CA), A 3 ft wide nonplanted area separated plots from each other. The treatments evaluated in this study included: a) Azdirachtin (40.0 oz/acre); b) *Bacillus thuringiensis*, subsp. Kurstaki (Btk), strain ABTS-35 (1.0 lb/acre); c) V-10433 (11 oz/acre); d) Spinosad (4 oz/acre); e) *Bacillus thuringiensis*, subsp. *aizawai*, Strain ABTS-1857 (1 lb/acre) with Pyriproxyfen (5 oz/acre); and f) an untreated control. We used a randomized complete-block design with four replications of each treatments. Insecticides were applied on four dates (10 Apr., 17 Apr., 25 Apr., and 30 Apr.). Applications of insecticides were made using a backpack sprayer with two D-4 hollow cone nozzles/bed delivering 70 gal/acre at 30 psi. We evaluated the insecticide treatments and untreated control by counting larvae and pupae from five randomly selected plants from each treated beds 48 h after chemical application.

Second study. This study was conducted in an experimental field of the Tropical Research and Education Center, Homestead, FL, starting in Feb. 2020. The preparation of the beds, experimental design and plant materials were similar as mentioned earlier under the "first study." The various treatments used in this study included: a) Spear (GS-omega/Kappa-HxTx-Hv1a) (1 pt/acre) in combination with BioProtect [3-(trihydroxysilyl) propyldimethyloctadecyl ammonium chloride] (1 pt/acre); b) Spear (GS-omega/Kappa-HxTx-Hv1a) (2 pt/acre) in combination with BioProtect [3-(trihydroxysilyl)propyldimethyloctadecyl ammonium chloride] (1 pt/acre); c) BioProtect [3-(trihydroxysilyl)propyldimethyloctadecyl ammonium chloride] (1 pt/acre); d) Spinosad (5 oz/acre); and e) an untreated control. Each treatment was replicated 4 times in a randomized complete block design. Applications of insecticides were made using a backpack sprayer with two D-4 hollow cone nozzles/bed delivering 70 gal/acre at 30 psi. Treatments were applied five weeks after transplanting cabbage. Evaluations of treatments was made by thoroughly checking five randomly selected plants/plot for diamondback moth life stages (larvae and pupae) 48 h after chemical application each week for 14 weeks.

Third study. This study was conducted in the TREC research plots. The plant materials were planted and maintained by following the similar experimental design and cultural practices mentioned earlier under "first study." Treatments evaluated in this study included: a) Spear (GS-omega/Kappa-HxTx-Hv1a) (1 pt/acre) with *Bacillus thuringiensis kurstaki* (1 pt/acre); b) Spear (GS-omega/Kappa-HxTx-Hv1a) (2 pt/acre) with *Bacillus thuringiensis kurstaki* (1 pt/acre); c) *Bacillus thuringiensis kurstaki* (1 pt/acre); d) Spinosad (4 oz/ acre); and e) an untreated control. Methylated seed oil at 0.125% v/v was added to each treatment. A randomized complete-block design was used with each treatment replicated four times. Treatments were applied 5 weeks after transplanting. All insecticides were applied once in a week and continued for six weeks. We used a backpack sprayer with two D-4 hollow cone nozzles/ bed delivering 70 gal/acre at 30 psi. Treatments were evaluated 48 h after each weekly application by checking five randomly selected plants/plot for diamondback larvae and moths.

Fourth study. In this study, 'Rio Verde' cabbage seedlings were transplanted on raised beds on 2 Feb. 2020, at the Tropical Research and Education Center, Homestead, FL. The experimental design, land prepation and maintenance of the plant were same as mentioned earlier under "first study." Different treatments used in this study includes: a) *Bacillus thuringiensis*, subsp. *aizawai*, Strain ABTS-1857 (1.0 lb/acre); b) *Chromobacterium subtsugae* strain PRAA4-1^T WDG (1 lb/acre); c) *Chromobacterium subtsugae* strain PRAA4-1^T WDG (2 lb/acre); d) *Chromobacterium subtsugae* strain PRAA4-1^T S30(1 lb/acre); e) *Chromobacterium subtsugae* strain PRAA4-1^T S30 (2 lb/acre); f) *Chromobacterium subtsugae* strain PRAA4-1^T (1 qt/acre); g) *Chromobacterium subtsugae* strain PRAA4-1^T (2 qt/acre); and h) an untreated control. Treatments were applied on three dates 4, 11, and 23 Mar. 2020 delivering 50 GPA per treatment using a backpack sprayer at 25–30 psi. A pre-count of insects was done on 2 Mar. before insecticide applications began. Evaluation of treatments for controlling diamondback moth was made by counting all larvae and pupae on randomly selected five plants in each treatment plot on three dates: 6, 13, and 23 Mar., 48 h. after each application of insecticides.

Statistical analysis. Data on the abundance of the various development stages of diamondback moth were transformed using square-root of $X + 0.25$ before performing an analysis of variance (ANOVA). The transformed data were analyzed by least squares ANOVA (PROC GLM, SAS Institute, 1989). However, for ease of interpretation, the means of the original data are presented in the table.

Results and Discussion

In the first study, five treatments were evaluated. Among those, the insecticides Spinosad and *Bacillus thuringiensis*, subsp. *aizawai*, Strain ABTS-1857 with pyriproxyfen was the most effective insecticides to control DBM larvae compared to the untreated control (Fig. 1). In both treatments the lowest number of DBM larvae was observed on the last sampling date. On the 2nd, 3rd, and 4th sampling dates, the DBM larval population was observed to be significantly low than untreated control except for the treatment with V-10433 (11 oz/acre). The DBM pupal population was found to be significantly lower in all treated cabbages than the untreated control (Fig. 2). The insecticide V-10433 did not differ from the untreated control in respect to the mean number of pupal populations.

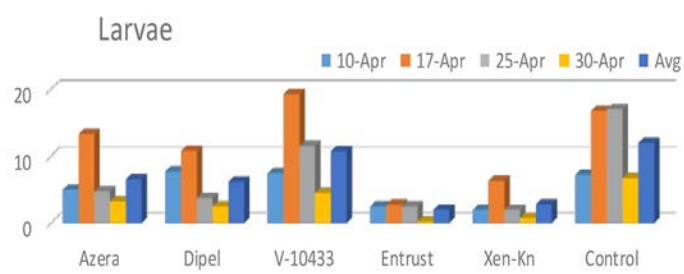

Fig. 1. Mean number of DBM larvae on different sampling dates treated with various reduce-risk insecticide and untreated control

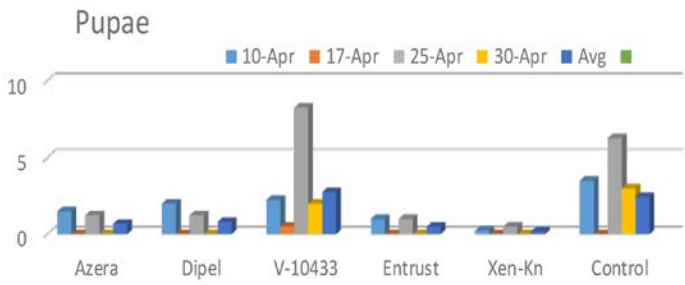

Fig. 2. Mean number of DBM pupae on different sampling dates treated with various reduce-risk insecticide and untreated control

In the second study, we observed the DBM larval and pupal population over 14 sampling dates. In the beginning of sampling, both larval and pupal populations were very low (Fig. 3). The larval population of DBM increased after the 3rd sampling date peaking on the 12th sampling date as can be seen in the untreated control. We observed a sharp decline of larval DBM on the 13th sampling date, but there was a sharp increase in the untreated control on the 14th sampling date. Both 3-(trihydroxysilyl) propyldimethyloctadecyl ammonium chloride (1 pt/acre) and Spinosad (5 oz/acre) significantly reduced the larval population compared to the other insecticide treatments the and untreated control. The GS-omega/Kappa-HxTx-Hv1a (1 pt/acre) in combination with 3-(trihydroxysilyl)propyldimethyloctadecyl ammonium chloride (1pt/acre) and GS-omega/Kappa-HxTx-Hv1a (2 pt/acre) in combination with 3-(trihydroxysilyl)propyldimethyloctadecyl ammonium chloride (1pt/acre) also showed a significant reduction of larval DBM compared to the untreated control. We observed an increase in pupal population in the untreated control from the 4th to the 8th sampling date (Fig. 4). Then the pupal population declined up to the 11th sampling date and increased again. Like the larval population, the pupal population was observed significantly lower in the 3-(trihydroxysilyl)propyldimethyloctadecyl ammonium chloride (1pt/acre) and Spinosad (5 oz/acre) treated cabbages compared to the other insecticide treatments and the untreated control. The GS-omega/Kappa-HxTx-Hv1a (1 pt/acre) in combination with 3-(trihydroxysilyl)propyldimethyloctadecyl ammonium chloride (1pt/acre) and GS-omega/Kappa-HxTx-Hv1a (2 pt/acre) in combination with 3-(trihydroxysilyl)propyldimethyloctadecyl ammonium chloride (1pt/acre) significantly reduced the pupal population than the untreated control throughout the sampling dates.

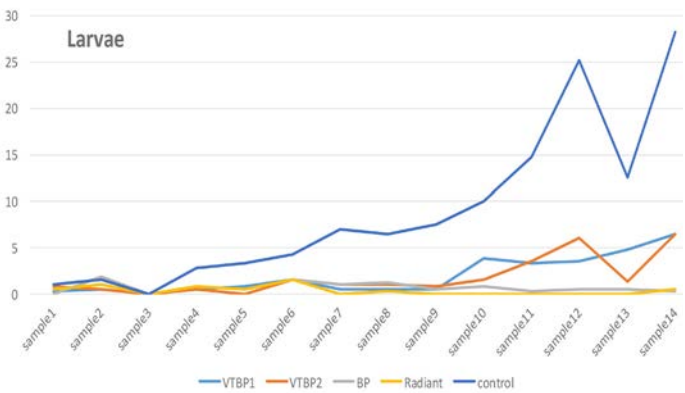

Fig. 3. Mean number of DBM larvae on different sampling dates treated with various reduce-risk insecticide and untreated control

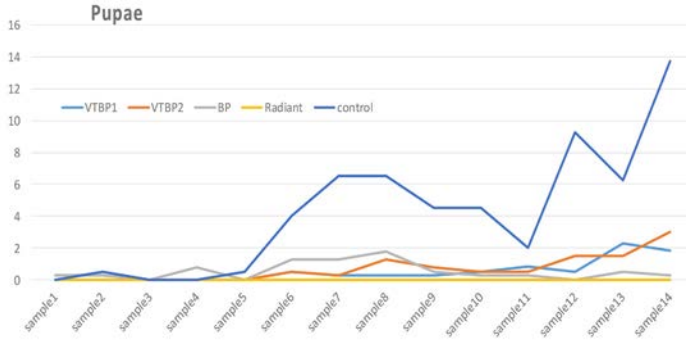

Fig. 4. Mean number of DBM pupae on different sampling dates treated with various reduce-risk insecticide and untreated control

In the third study, we observed significantly more DBM larvae in the untreated control than in treated cabbages for the first three sampling dates (Fig. 5). The larval population showed an increase after the 4th sampling date. The larval population was significantly lower in Spinosad treated cabbages on the last sampling date compared to the other insecticide treatments and the untreated control. Other than Spinosad, GS-omega/Kappa-HxTx-Hv1a (1 pt/acre) with *Bacillus thuringiensis kurstaki* (1 pt/acre) and GS-omega/Kappa-HxTx-Hv1a (2 pt/acre) with *Bacillus thuringiensis kurstaki* (1 pt/acre) reduced the larval DBM population more on the last sampling date (6 May, 2020) than the other insecticide treatments and the untreated control. The pupal population increase sharply from the 3rd (16 Apr. 2020) to the 5th sampling date (30 Apr. 2020) in all treated and untreated cabbages (Fig 6). On the 5th sampling date, Spinosad significantly

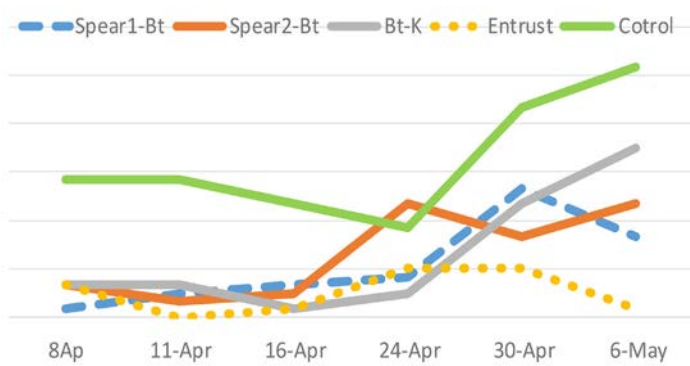

Fig. 5. Mean number of DBM larvae on different sampling dates treated with various reduce-risk insecticide and untreated control

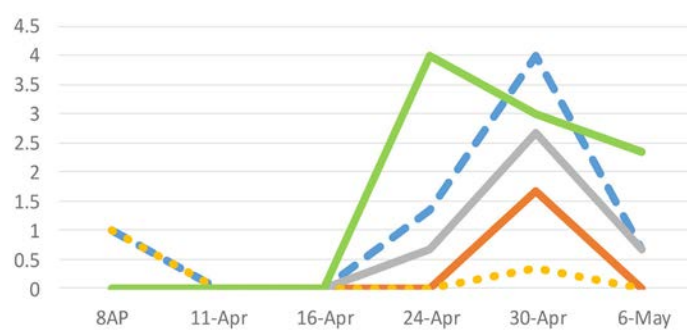

Fig. 6. Mean number of DBM pupae on different sampling dates treated with various reduce-risk insecticide and untreated control

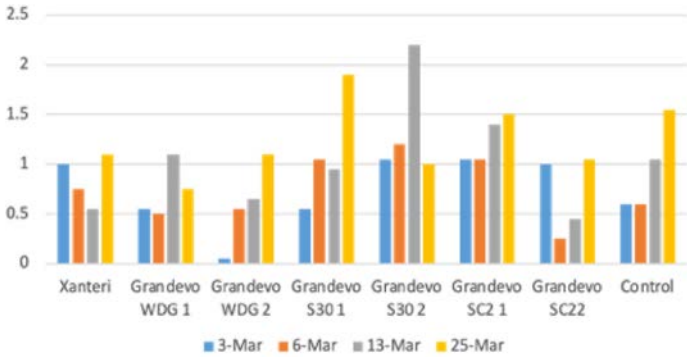

Fig. 7. Mean number of DBM larvae on different sampling dates treated with various reduce-risk insecticide and untreated control.

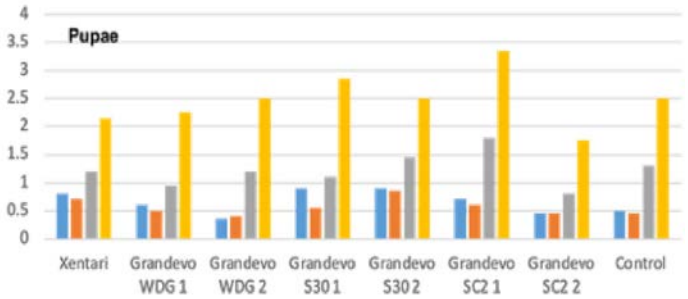

Fig. 8. Mean number of DBM pupae on different sampling dates treated with various reduce-risk insecticide and untreated control.

reduced the pupal population compared to the other insecticides and the untreated control.

In the fourth study, on the pre-spray sampling date (2 Mar.), the mean number of DBM larvae/plant treated with various insecticides did not differ from the untreated control (Fig. 7). On the first post-spray sampling date (6 Mar.), *Chromobacterium subtsugae* strain PRAA4-1^{T} (2 qt/acre) had fewer larvae/plant than any other treatment but was not significantly different from the untreated control. On the second post-spray sampling date (13 Mar.), insecticide treatments did not differ from the untreated control for the mean number of larvae. When means across the sampling dates are averaged, the mean number of diamondback moth larvae was lowest in *Chromobacterium subtsugae* strain PRAA4-1^{T} WDG (2 lb/acre) treated plants than other treatments including the untreated control. Mean number of pupae/plants treated with various treatments varied on different sampling dates (Fig. 8). However, no consisted trend was observed in the effectiveness of insecticides in reducing DBM pupae. Overall, the mean number of pupae in the various treatment on different sampling dates did not differ from the untreated control.

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Biology and Abundance of Cowpea Weevil, on Different Leguminous Seeds

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Additional index words. Bean weevil, legumes, development period, sex ratio

Cowpea weevil (*Callosobruchus maculatus*) is an important stored grain pest of leguminous crops in tropical and sub-tropical regions of the world. It is also used to rear the biocontrol agent, *Catolaccus hunteri*, of pepper weevil (*Anthonomus eugenii*) under laboratory conditions. The fecundity and survival of cowpea weevils can be different on various legume seeds. Experiments were conducted in a growth chamber to determine the host preference of cowpea weevil using five different seeds including mung (*Vigna radiate* (L.) R. Wilczek), chickpea, a.k.a garbanzo bean (*Cicer arietinum* L.), black bean (*Phaseolus vulgaris* L.), black-eyed pea (*Vigna unguiculata* L. Walp.), and kidney bean (*Phaseolus vulgaris* L.). The survival rate and developmental time of cowpea weevil on these seeds was evaluated. Parameters evaluated were: fecundity, life cycle, oviposition period, sex ratio, and developmental time. Newly emerged pairs of adults were introduced to Petri dishes, each containing 20 g of seeds. Each petri dish had one pair of adult weevils. Each treatment was replicated three times. The weevils were left in the petri dishes until they died. The number of eggs laid was counted on each type of seed at two-day intervals. The emergence of adults and the sex ratio was recorded on the different legume seeds.

Cowpea weevil, *Calosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae) is an important pest of legume seeds (Beck and Blumer, 2014). It originated in Asia and Africa and later spread to tropical and sub-tropical parts of the world (Beck and Blumer, 2014). Each female has the potential to lay more than 100 eggs during its lifetime (Beck and Blumer, 2014). Eggs are glued to the surface of seeds. When the egg hatches it produces frass and the egg shell becomes opaque (Devereau et al., 2003). The larva bores inside the seed and feeds on it. The larva turns into a pupa. Pupation is marked by thinning of the seed shell (Beck and Blumer, 2014). The adult chews on the seed and emerges. The male and female of cowpea weevils show distinct polymorphism. The female is characterized by having a large black plate at the end of the abdomen with a white longitudinal strip, whereas the male plate is smaller and lacks this strip (Beck and Blumer 2014). The life cycle is completed in 3–4 weeks (Beck and Blumer, 2014).

The generation time depends on the host, temperature and relative humidity (Howe and Currie, 1964; Schoof, 1941). The larvae cause the major damage to seeds. Cowpea weevils can cause 100% damage to stored grains in six to nine months if left unchecked. They can also cause weight loss in seeds of up to 60% (Rees, 2004). It is estimated that the economic losses caused by cowpea weevil infestation in stored grain legumes are 35%, 7 to 13%, and 73% in Central America, South America, and Kenya respectively (Nahdy, 1994; Hu et al., 2009; and Tanzubil, 1991).

Cowpea weevil is used for rearing of *Catolacus hunteri*, a biocontrol agent of pepper weevil (*Anthonomus eugenii*) (Seal et al., 2002; Vásquez et al., 2005). It is important to identify the hosts for rearing the cowpea weevil. There is also a need to determine the damage caused by cowpea weevil on different leguminous seeds. The objective of this study was to determine the fecundity, sex ratio, and oviposition period on cowpea weevil on five different legume hosts including mung beans, black beans, black-eyed peas, kidney beans, and garbanzo beans (chickpeas).

Materials and Methods

The study was conducted in a growth chamber in a laboratory at Tropical Research and Education Center, Homestead, FL. The growth chamber was maintained at 30 °C, 70% relative humidity with a daily light regime of 14/10 h (light/dark) The treatment included five leguminous hosts: mung bean, black bean, black-eyed peas, kidney beans, and garbanzo beans (chickpea) (Fig.1). Mung beans were bought from Oh nuts (Hollywood, Fla), garbanzo beans from Rani (Houston, TX), black-eyed peas and lidney beans from Goya (Secaucus, NJ) and black beans from Iberia (Brooklyn, NY). Prior to initiating the study, seeds of each crop (treatment) were washed with 10% Clorox to remove any preservatives. Each treatment was replicated three times. For each replicate, 20 g of seeds were kept in a Petri dish (5 mm diam). and one pair of newly emerged (0–4 h old) male and female cowpea weevils was placed in each Petri dish. Eggs were counted and separated every other day until the male and female died in each Petri plate. After removal of the infested seeds, the same number of similar fresh seeds were added to the Petri plate.

The pre-oviposition, oviposition and post-oviposition periods were also recorded for newly (0–4 hr–old) emerged females.

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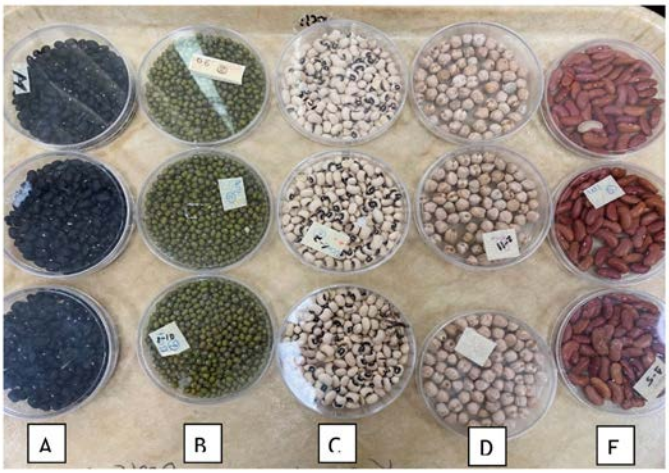

Fig. 1. Treatments: A) black beans B) mung bean C) black-eyed peas D) garbanzo E) kidney beans

To estimate the pre-oviposition period, the seeds on the verge of adult emergence were kept separately in glass vials (25 mm) with a loose cap to allow air movement. Immediately (0–2 h) after the adult emerged, one male and female were removed from the vials and kept in a Petri dish containing mung beans to note the pre-oviposition period. The seeds were checked carefully at 5-minute intervals using a hand lens to record the time that the first egg appeared. The same procedure was repeated five times.

For the sex ratio experiment, eggs were separated from all the replicates every other day and placed separately in a Petri dish and marked with the date of oviposition and seed type. At the end, we had six petri plates for each treatment, each with eggs of same age group which were placed in an incubator at 29 ·C to observe insect development. Starting 18 d after the eggs were collected, the Petri dishes were checked at 24-h intervals and all emerged adults were collected. The number of males and females that emerged were removed from the Petri dish and the sex ratio was determined. At the same time, the number of days required for each adult to develop on each host was also recorded. The total development period was also estimated on each host by observing the time from egg laying to adult emergence.

The total number of eggs and the total number of emerged adults were recorded for each treatment to determine the percentage of adults which emerged with respect to the number of eggs laid.

Statistical analysis. Data on the oviposition, adult emergence, sex ration recorded from host samples, were transformed using square-root of $X + 0.25$ before performing an analysis of variance (ANOVA). The transformed data were analyzed by least squares ANOVA (SAS Institute Inc. 2013). However, for ease of interpretation, the means of the original data were presented in the table. The Waller-Duncan k ratio test was used to separate treatment means where significant ($P < 0.05$) differences occurred [Waller Duncan K-ratio test ($\alpha < 0.05$) using SAS (SAS Institute Inc. 2013).

Results

Fecundity. We observed a significant difference in the number of eggs laid on each host. The maximum fecundity was recorded on mung beans followed by black beans, black-eyed peas, kidney beans and the least on garbanzo beans (Fig. 2). The number of eggs laid was not significantly different among mung bean, black bean, black eyed peas and kidney beans. The fecundity increased and reached the peak and as the female aged, withthe number of eggs laid decreasing and eventually stopping until the female died (Fig. 3).

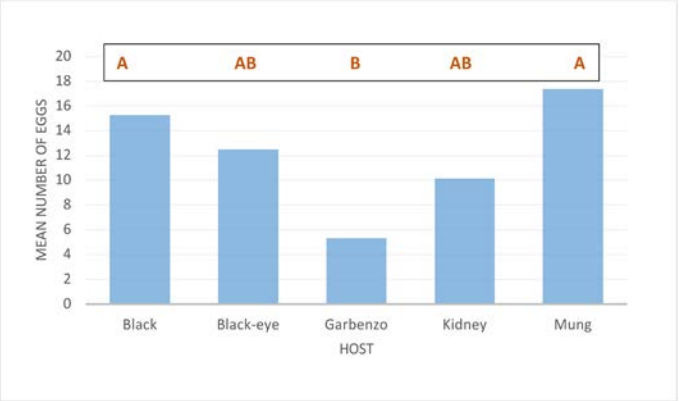

Fig. 2. Mean number of eggs/female of *Callosobruchus maculatus* on different leguminous seeds. Bars represent the mean of three replicates.

Adult emergence. Maximum adult emergence was in mung beans followed by black-eyed peas and garbanzo beans (Fig. 4). We did not find any adult emergence in black beans or kidney beans because the development of cowpea weevil was not completed on

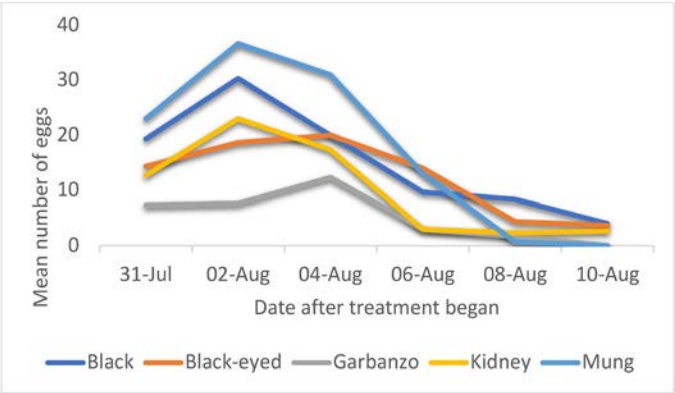

Fig. 3. Mean daily fecundity of *Callosobruchus maulatus* on different hosts. Lines indicate the means of three replicates.

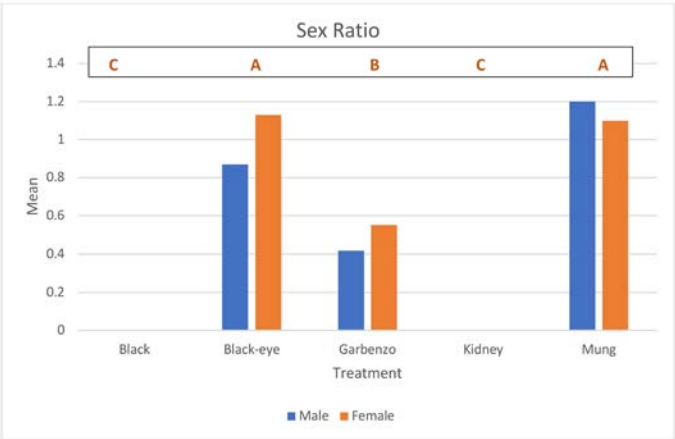

Fig. 4. Mean number of Male and Female (sex ratio) of *Callosobruchus maculatus* F_1 progeny/female on different hosts. Mean of six replicates.

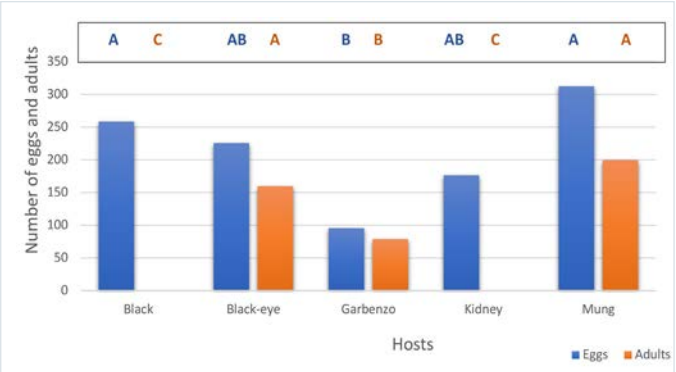

Fig. 5. Mean number of eggs and F_1 adults/female of *Callosobruchus maculatus* on each host; mean of three replicates.

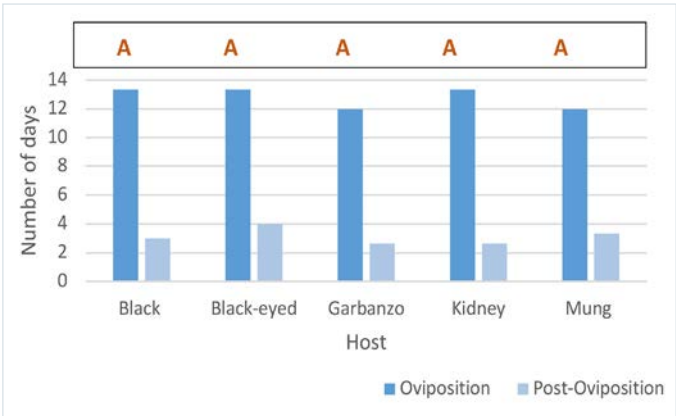

Fig. 6. Oviposition period of *Callosobruchus maculatus* on different hosts.

either black beans or kidney beans. In mung beans, the number of males was higher than the number of females while the opposite was observed in black-eyed peas and garbanzo beans where the number of females was higher than the number of males.

Sex ratio. We observed a significant difference in the mean number of males and females emerged in each host. The highest number of male and female progenies were observed on mung bean, black-eyed peas and garbanzo bean, respectively (Fig 5).

We observed that as long as the female mates, she starts laying eggs. We estimated the pre-oviposition period to be 0–2 h. The oviposition period was similar in each host, lasting from 12–14 d followed by a post-oviposition period of 3–4 d (Fig. 6). The percentage of eggs laid to adult emergence was highest in garbanzo beans followed by black-eyed peas and mung beans. The mean development period varied among different hosts. The development period was shortest in black-eyed peas followed by mung beans and highest in garbanzo beans (Fig. 7).

Discussion

This study showed that the development time, sex ratio and, fecundity of the cowpea weevil depends on the host. We conclude that all legume seeds are not feasible as host seeds for the cowpea weevil to complete its life cycle. We believe that non-completion of development in black beans and kidney beans can be due to the physiochemical properties of the seed or due to thickness of the seed coat. Hudaib (2019) found that the thickness of seed coat

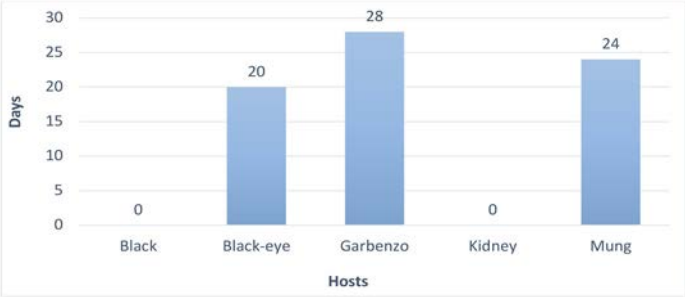

Fig. 7. Mean development period of *Callosobruchus maculatus* on different hosts.

of kidney bean is four times greater than that of black-eyed peas. This may have made kidney bean resistant to cowpea weevil. Seed coats are a barrier for penetration of two bruchid species *Callosobruchus chienesis* and *Callosobruchus maculatus* in *Vicia faba* (broad bean). Only 45 to 58% of larvae were able to reach the cotyledons (Boughdad et al., 1986; Desroches et al., 1995). Chemicals present in the seed coat can have a detrimental effect on the survival of cowpea weevil (Hudaib et. al. ,2017). Kidney beans contain phenolic compounds and terpinoids which have found to contain insecticidal properties (Rattan 2010). Phenolic compounds can also reduce oviposition in bruchid beetle, *Callosobruchus chinensis* (Upasani and Kotkar 2003; Salunke 2005). The presence of toxic chemicals in the seed coat of *Canavalia ensiformis* (jack bean) hinders the development and life cycle of cowpea weevil (de Sá et al., 2018). Moisture content plays a very important role in the development of an insect. Carbohydrate, protein, fat and moisture content were positively correlated with orientation of cowpea weevil towards mung beans (Yewale and Kadam 2020). In the present study, we observed frass in the egg shell on the infested seeds of black beans and kidney beans. This indicated that the egg hatched but did not turn into an adult. For future research, we will try to determine the exact reason for non-completion of development in order to develop resistant cultivars.

We have found that for rearing the biocontrol agent *Catolaccus hunteri* (larval parasiotoid) of pepper weevil there is a need for two hosts for cowpea weevil. Rearing cowpea weevils on mung beans and black-eyed peas provide many adult weevils for egg laying in short time. However, for oviposition of the parasitoid, the host needs to remain in the larval stage for a longer time. We observed that on garbanzo bean, cowpea weevils not only remain in the larval stage for a longer duration, but also that if multiple eggs are laid on the garbanzo beans, the larvae tend to make holes in the seed which give them more potential exposure to the parasitoid.

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Application of Small Unmanned Aerial Vehicles to Improving Vegetable Crop Management

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Additional index words. crop management, disease, sUAV, multispectral sensing, vegetables

Small Unmanned Aerial Vehicles (sUAV) have potential advantages in the agricultural industry. This publication is to describe the use of sUAV in vegetable crop management detecting plant stress and how widely it is distributed. Examples include the detection of dead patches in a tomato field, plant death of yellow squash caused by anthracnose, and the distribution of Bean Golden Mosaic Virus. The devices used are a DJI Phantom 4 Pro with a preinstalled High Definition (HD) camera and a multispectral sensor—Sentera double 4k specifically for agriculture. The autonomous flight setup with appropriate parameters was used to cover the targeted fields. The results showed that in a single flight, multiple images including color, Red, Green and Blue (RGB), Near-Infrared (NIR), Normalized Difference Vegetation Index (NDVI), and Normalized Difference Red Edge (NDRE) were collected simultaneously. With software designed for this purpose, flight data were processed to generate various images based on plant health status and damage caused by diseases. This technology can provide timely information to vegetable growers so they can manage their crops and minimize yield losses.

The use of a small Unmanned Aerial Vehicle (sUAV), also called small Unmanned Aerial System (sUAS) or simply called a drone, has shown the ability to change many aspects of human life. With rapid development of artificial intelligence (AI), the implementation of sUAV systems has been applied to agriculture with labor cost savings and real time information collection to assist growers. It has been used in many surveys and to create spatial mapping related to soil properties (Abbas et al., 2013) and crop health, such as estimating plant canopy (Mathews and Jensen, 2013), detecting plant diseases (Johansen et al., 2014; Wang et al., 2020; Wang and Zhang, 2021), evaluating intact crop biomass (Wang et al., 2019) and estimating plant nutrient levels (Bendig et al., 2014; Vega et al., 2015).

The effective use of an sUAV ultimately depends on the type and model of the device and a setup with appropriate flight parameters, and data collection and processing. For instance, a high definition (HD) camera preinstalled on a sUAV can take a lot of high-quality colorful photos across an entire field based on its size, flight height, speed, and overlaps between any two photos. Color is a combination of three spectrum bands—red, green, and blue (RGB). All these individual photos need to be stitched together to generate an integrated spatial image, called RGB. In addition to the HD camera, a specific sensor is needed because the human eye can only see a very narrow band of the light spectrum with a wavelength of 400–700 nm. A Near-Infrared (NIR) or thermal sensor with the wavelength beyond 700 nm can easily detect plant health status based on plant chlorophyll content. Therefore, NIR sensors have been developed and are widely used in agriculture. However, a NIR image is black and white in color. To improve its visibility, some multispectral sensors have been developed and combined with RGB to generate colorful images, i.e., Normalized Difference Vegetation Index (NDVI), and Normalized Difference Red Edge (NDRE). Similarly, the individual images of NIR, NDVI or NDRE captured or derived from separate sensors need to be stitched together to form a single mosaic mapping image with software such as FieldAgent™. To use an sUAV system in a local vegetable setting, this paper gives some examples of applications in vegetable fields which can be used to improve crop management.

Materials and Methods

An sUAV system with a model DJI Phantom 4 Pro with a HD camera and double 4k multispectral sensor (Sentera, MN) preinstalled (Fig. 1, top) was used in field surveys for various vegetable crops from 2018–19 across a major agricultural area the Redland in southwest Miami-Dade County (Fig.1, bottom). The vegetable crops included in these surveys were snapbeans, squash, and tomato. Field size ranged from 20 to 40 acres. The program FieldAgent (Sentera, MN) can be downloaded to either an iPhone or an iPad. Then an autonomous flight was set up for each field with appropriate parameters, such as picture overlap 80%, flight altitude 200–400 ft above the ground, travel speed 10–20 miles per hour (mph), and an orientation based on the wind direction, the objective of the survey, crop type, growth stage, and type of stresses. Once each corner of the field was marked with Google Maps on the mobile device, the system would display the field size, the number of images would be collected, the number of batteries needed, the duration of flight time, the picture pixel size, and Ground Survey Distance (GSD) in inches (in) or centimeters (cm) per pixel (Wang, 2019).

To understand the kind of stress(es) caused the dead patch, the surveyor has to zoom in on the particular spot or to con-

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Fig. 1. The sUAV system (top) applied (Sentera, MN) and the distribution of field surveys across the major agricultural area of Miami-Dade County (bottom) in the last a couple of years.

duct a physical ground survey. For instance, the ground survey showed that the dead patch in Fig. 2 was caused by a physical or mechanical practice because the plants on the left side of the field had been harvested once. That is why the plants on the right side of the block looked greener and better than those on the left (Fig. 2). By using the same technology, the distribution pattern of tomato chlorotic spot virus (TCSV) was detected in another field (Wang et al., 2020).

Field survey on squash. Based on an NDVI mosaic image derived from the sUAV system from a squash field, one can tell that plants were not growing uniformly because of the many yellow (dying) and red (bared ground) dots distributed on the image except for field edges and driveways across the field (Fig. 3 left). A close look at the RGB image from the same spot showed that many plants had died in this field (Fig. 3 right). A further investigation with sampling and diagnosis showed that the dead plants were caused by anthracnose, which is related to excess rainfall or irrigation water.

Similarly, some dead patches caused by phytophthora plant rot in yellow squash which were caused by excess rain with water logging in patches because of uneven land (Wang and Zhang, 2021) were identified using sUAV. Laser levelling of the land before growing yellow squash to prevent water-logging would

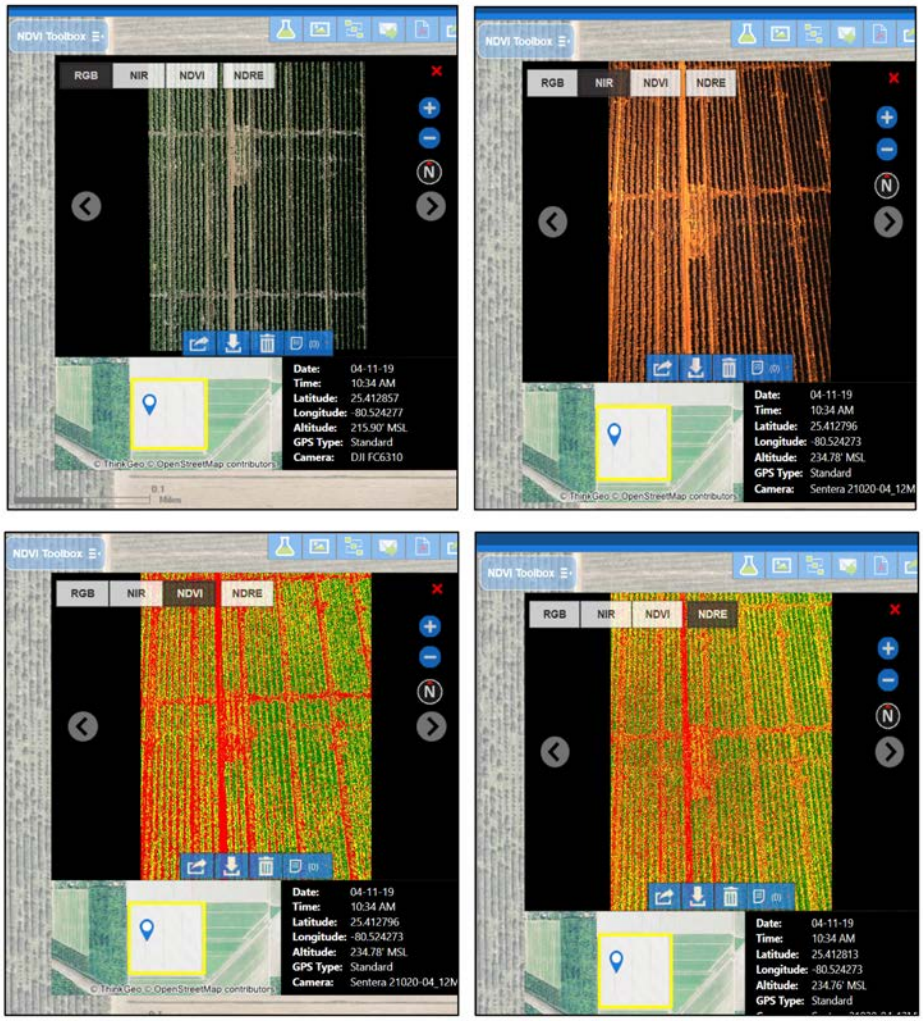

Fig. 2. A series of mosaic images (from top left: RGB, NIR, NDVI, and NDRE) of a surveyed tomato field with a dead patch.

be helpful, (though it may not be possible due to the nature of the unusual soil in southern Miami-Dade). Growers can implement Integrated Pest Management (IPM) strategies to prevent and suppress fungal diseases, especially anthracnose and phytophthora blight, in a timely using cultural practices and by following Vegetable Production Handbook of Florida guidelines (Dittmar et al., 2020).

Incidence of Bean Golden Mosaic Virus (BCMV). Using the same techniques, a snapbean field was surveyed with the sUAV system, which showed a severe incidence of BGMV (Fig. 4).

BGMV is a major problem in snapbeans. It causes yellowing on young leaves first and may severely reduce yield. A pre-bloom infection may cause yield losses of up to 90% (Zhang et al., 2017). Insects such as thrips and whiteflies, especially the silver-leaf whitefly, are vectors for BGMV and can easily transfer the virus from infected plants to healthy ones. The disease is usually worse during the early or late growing season. Control of whiteflies by using IPM tactics is a key to controlling the spread of this virus.

Pest management is always critical and challenging for vegetable growers in a subtropical region because the pests can live and reproduce year-round. Implementing IPM is a fundamental guideline that includes cultural, genetical, biological, mechanical and physical control measures. However, identifying the pests and the damage level on time is critical to taking action. An sUAV system allow rapid collection of real-time information with a system that is cost saving, and provides reliable results to help vegetable growers make decisions to minimize the yield losses.

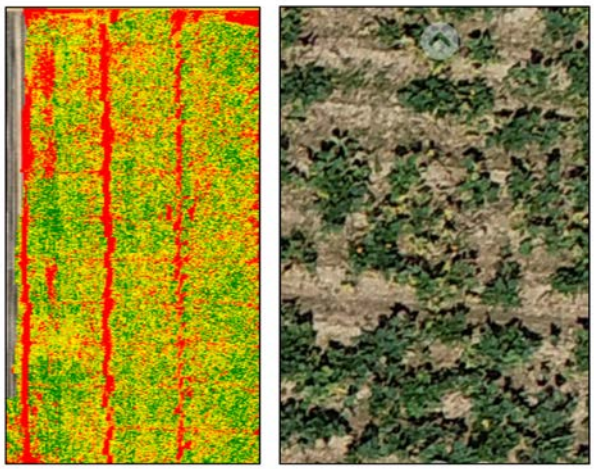

Fig. 3. NDVI image from a yellow squash field (left) with healthy (green) and unhealthy (red and yellow colors) plants (right).

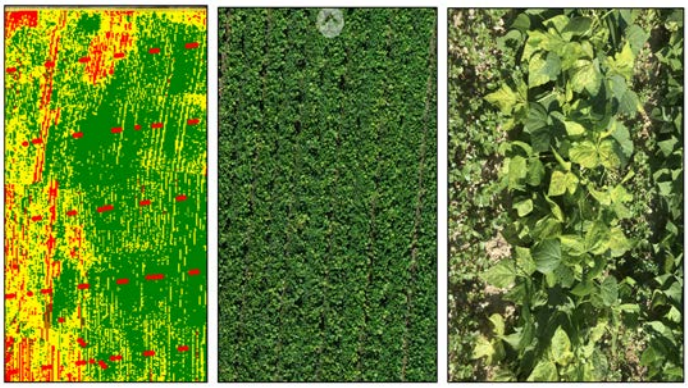

Fig. 4. The NDVI image (left, the red dots indicating flight pattern and photographs taken), the RGB image (middle), and a close look on symptoms of bean gold mosaic virus (right).

Conclusion

The use of an sUAV system with related AI technology allows field surveys to be carried out with rapid precision and reliable results. The information collected through the field survey and data processing may provide growers so they can make timely decisions to reduce the yield and quality losses. With the great potential and rapid development of the AI technology, more and more applications of the sUAV systems in agriculture will be available in the future.

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Effect of IN-M1 Concentric® on Plant Growth and Fruit Yield of Tomato In South Florida

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Additional index words. biostimulant, tomato, IN-M1, garden solution, fertilizer rate

The effect of a biostimulant, IN-M1 Concentric®, in promoting plant growth and yield of tomato was evaluated under field conditions in Homestead, FL, over two growing seasons. 'Sanibel' tomato was used both seasons. All treatments were arranged in a randomized complete block (RCB) design with six replications for each treatment. In the 2017–18 season, IN-M1 was first applied at 1% by soil drench at transplanting and then as a foliar spray at flowering. Four fertilization levels, 25%, 50%, 75%, and 100% of the recommended rate were applied through irrigation drip lines. No significant increases in fruit yield were detected in any treatments compared to the untreated control. However, a beneficial effect on fruit yield was found with 75% fertilization level, where total yield from the first two harvests increased by 5.1%. In the 2018–19 season, IN-M1 was applied through irrigation drip immediately after transplanting and at flowering. Three rates of IN-M1 [0.25, 0.5, and 1.0 gallons/acre (gal/A)] were tested on tomato plants grown at 90% of the recommended fertilization rate. IN-M1 at 0.5 gal/A significantly ($P < 0.1$) increased plant height compared to the untreated control. Though the effect of IN-M1 on fruit yield was not significant at ($P = 0.1$) compared to the untreated control, beneficial effects on fruit yield were observed. IN-M1 at 0.25, 0.5, and 1.0 gal/A increased yield of extra-large and large sized fruit by 12.7%, 18.3%, and 9.3%, respectively, compared to the untreated control. The biostimulant IN-M1 could be applied to promote plant growth, increase fruit yield, and reduce fertilizer use in tomatoes in south Florida.

Florida ranks first in the production of winter fresh market tomato in the United States (Tomato101). Florida provides more than 50% of fresh tomatoes in the country on 31,500 acres. The total value of tomato exceeds $600 million, which accounts about one third of the total value of all fresh vegetable crops in Florida.

Tomato plants in open field conditions often face various stresses in Florida at different developmental stages, including transplanting shock, heat, chilling, etc. One approach to coping with such environmental stresses is to apply biostimulants (Bulgari et al., 2019; Colla and Rouphae, 2015). Plant biostimulants are products prepared from different organic or inorganic substances and/or microorganisms, that have beneficial effects on improving plant growth, productivity, and alleviating negative effects of abiotic stresses (Bulgari et al., 2019).

IN-M1 Concentric® is a plant biostimulant and registered as 'Garden Solution' in the US. It is a mixture of beneficial microorganisms and their metabolites <http://www.concentricag.com/>. IN-M1 has been applied in high value vegetable crops, such as lettuce, strawberry and fresh market tomato to promote plant growth and fruit yield. IN-M1 contains seven beneficial bacterial species and three fungal species. It also has been reported that IN-M1 can be used to reduce fertilizer rates, particularly nitrogen, in vegetable crops. In this study, we conducted two field trials to evaluate the beneficial effects of IN-M1 on tomato production in south Florida: 1) effect of IN-M1 on tomato fruit yield and reducing fertilizer use during 2017–18 and 2) effect of IN-M1 rate on tomato plant growth and fruit yield during 2018–19.

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Materials and Methods

Field preparation and crop management. The field trials were conducted over two tomato growing seasons (2017–18 and 2018–19) at the University of Florida's Tropical Research and Education Center, Homestead, FL. Tomato (cv. Sanibel) seedlings were transplanted in beds covered with black plastic mulch in both growing seasons. Irrigation and fertigation were provided by two drip irrigation tapes passing on both sides of each plant, following guidelines from the Vegetable Production Handbook of Florida, 2017–18 (Ditmar et al, 2017). Standard spray programs developed in the lab were followed to control common diseases and pests on tomato.

Effect of IN-M1 on tomato fruit yield and reducing fertilizer usage. A field trial was conducted in the 2017-2018 growing season to evaluate the effect of IN-M1 on tomato fruit yield and reducing fertilizer usage. Each plot consisted of a 22-ft section on a single bed with a 2-ft buffer zone between adjacent plots. Within each plot, 11 plants were planted 2-ft apart. Each bed received one of four levels of fertilization, 100%, 75%, 50%, and 25% of the recommended rate from the Vegetable Production Handbook of Florida 2017–18 (Ditmar, et al., 2017). After transplanting, liquid fertilizer 3–0–10, was applied to each bed twice a week throughout the growing season at the specified rate. Plots treated with IN-M1 and untreated plots were arranged randomly on each bed, with six replicates per treatment.

Tomato seedlings were transplanted into beds on 7 Dec. 2017. After transplanting, seedlings were drenched with Danitol (fenpropathin) at 3.0 mL/gallon to protect seedlings from damage by cutworms. IN-M1 was applied at 1% (v:v) as a soil drench at the base using about 120 mL /plant right after transplanting. A boost

Table 1. Effect of IN-M1 on tomato yield at four fertilizer levels (2017–18).

	Recommended fertilizer[y]							
	25%		50%		75%		100%	
Yield per acre (lb)[z]	CK	IN-M1	CK	IN-M1	CK	IN-M1	CK	IN-M1
1st harvest	9503 a	8385 a	10062 a	9743 a	11660 a	12059 a	8146 a	9503 a
2nd harvest	12618 a	14055 a	19326 a	18528 a	19885 a	21083 a	20524 a	20205 a
3rd harvest	6229 a	6469 a	10062 a	9264 a	14614 a	13177 a	14295 a	10861 a
Extra large	12698 a	12698 a	20045 a	18767 a	22360 a	22680 a	20923 a	19087 a
Large	13416 a	14135 a	16052 a	16052 a	19326 a	19246 a	17809 a	17409 a
Medium	2236 a	2076 a	3354 a	2556 a	4472 a	4392 a	4153 a	4073 a
Total yield	28350 a	28909 a	39451 a	37451 a	46159 a	46318 a	42885 a	40569 a

[z]Plants population was estimated at 3660/acre with 2 ft between plants to estimate the fruit yield/acre.

[y]Means followed by the same letters in each row with each item for comparison at each fertilizer level indicate no significant difference at $P = 0.05$.

foliar application was applied at 1% on 10 Jan. 2018 when plants started flowering. Fruit were harvested from all plants in each plot on 26 Feb., 13 Mar., and 27 Mar. 2018. Fruit were graded into three categories: extra-large, large, and medium based on the USDA standard. Fruit yield of each category was recorded for each plot at each harvest. Student t-test was performed using the SAS statistical software Version 9.4 (SAS Institute Inc., Cary, NC). Means of fruit yield for each category were separated between the treatments and the untreated control at each fertilization level using Fisher's protected LSD ($P = 0.05$).

Effect of IN-M1 rate on tomato plant growth and fruit yield. A field trial was conducted in the 2018–19 growing season to evaluate the effect of IN-M1 rate on tomato plant growth and fruit yield. Each plot consisted of a 35-ft section on a single bed with a 2-ft buffer zone between adjacent plots. Within each plot, 17 plants were planted 2-ft apart. Treatments included IN-M1 at 1.0, 0.5, 0.25 gallons/acre and an untreated control. IN-M1 was applied through drip irrigation lines. The experimental design was a randomized complete block (RCB) design with six replications. Fertilizer (3–0–10) was applied twice a week after transplanting at 90% of the recommended rates according to the Vegetable Production Handbook of Florida 2018-2019 (Ditmar et al., 2018).

Tomato (cv. Sanibel) seedlings were transplanted on 30 Oct. 2018. After transplanting, seedlings were drenched with Danitol (fenpropathin) at 3.0 mL/gallon to protect them from damage by cutworms and other insects. IN-M1 was first applied right after transplanting on 30 Oct. and again on 26 Nov. 2018 when plants started flowering. Plant height was measured on 20 Nov. 2018. Fruit of the six best plants in each plot were marked and fruit were harvested from the selected plants on 30 Jan., 11 Feb., and 19 Feb. 2019. Fruit were graded into three categories by size: extra-large, large, and medium based on the USDAs grade standards. Fruit yield of each category was recorded for each plot at each harvest. Analysis of variance (ANOVA) was performed using the SAS statistical software Version 9.4 (SAS Institute Inc., Cary, NC). Means of plant height and fruit yield per plant for each category were separated among the treatments using Fisher's protected LSD ($P = 0.1$).

Results

Effect of IN-M1 on tomato fruit yield and reducing fertilizer usage. In this trial, treatments with IN-M1 did not have significant effect on tomato yield at any of the four fertilizer levels (Table 1), due to the large variation among replications. However, beneficial effects were observed with IN-M1 on increasing early fruit yield and total yield at 25% and 75% of recommended fertilizer levels, particularly yield of extra-large and large fruit, which increased by 719 lb/acre and 240 lb/acre, respectively (Table 1).

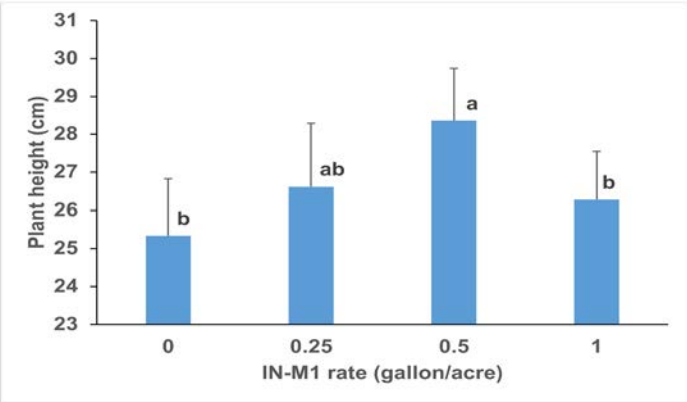

Fig. 1. Effect of IN-M1 at three rates on tomato plant growth in the 2018–19 growing season. Bars (with standard errors on the top) labeled with same letters were not significantly different at $P = 0.1$.

Effect of IN-M1 rate on tomato plant growth and fruit yield. Application of IN-M1 enhanced early plant growth when 90% of the recommended fertilizer was applied. IN-M1 at 0.5 gallon/acre significantly ($P < 0.1$) increased plant height compared to the untreated control at one month after transplanting (Fig. 1). In addition, plant growth looked more uniform when tomato plants were treated with IN-M1 at 0.5 and 1.0 gallon/acre. Though not significant at ($P = 0.1$) compared to the untreated control, IN-M1 at all three rates increased fruit yield (Table 2): of extra-large and large fruit by 9.3% to 18.3% and total yield by 11.0% to 16.9% (Table 3). Among the three rates tested, 0.5 gallons/acre was the best one for increasing plant growth and fruit yield.

Discussion and Conclusions

In our field trials conducted during two growing seasons, tomato plants which received IN-M1 treatment had better early growth and produced higher yields of extra-large and large fruit at early harvest. In addition, our results indicated that less fertilizer could be used to achieve a similar yield when plants were treated with IN-M1. Our results agreed with others from elsewhere with IN-M1 on vegetable crops including tomato.

It has been reported that application of biostimulants can help plants combat abiotic stresses including transplant shock, thus

Table 2. Effect of IN-M1 on tomato plant growth and yield (2018–19).

	Fruit yield /plant (kg)[z]				
Treatment	Extra-large	Large	Extra-large +large	Medium	Total
Untreated control	3.26 a	2.43 a	5.69 a	0.69 a	6.39 a
IN-M1 @ 0.25 gallon/acre	3.57 a	2.84 a	6.41 a	0.80 a	7.21 a
IN-M1 @ 0.5 gallon/acre	3.92 a	2.81 a	6.73 a	0.74 a	7.47 a
IN-M1 @ 1.0 gallon/acre	3.65 a	2.57 a	6.22 a	0.87 a	7.09 a
LSD ($P = 0.1$)	0.70	0.49	1.03	0.24	1.12

[z]Means followed by same letters in each row indicate no significant difference at $P = 0.1$.

Table 3. Effect of IN-M1 on tomato yield increase.

	% Yield increase	
Treatments	Extra-large + Large	Total
Untreated control	–	–
IN-M1 @ 1 gallon/acre	9.3	11.0
IN-M1 @ 0.5 gallon/acre	18.3	16.9
IN-M1 @ 0.25 gallon/acre	12.7	12.8

enhancing plant growth (Bulgari et al., 2019). In one of our field trials, tomato plants treated with IN-M1 at 0.5 gallon/acre were significantly taller than the untreated control (Fig. 1). Because IN-M1 is a mixture of beneficial microorganisms and their metabolites, it is difficult to define the exact mechanism(s) leading to such an effect on plant growth promotion. It has been suggested that the application of biostimulants can benefit nutrient uptake and efficiency (Bulgari et al., 2019; Colla and Rouphae, 2015), which may help achieve similar fruit yields with reduced fertilizer usage. This will not only help reduce the input for growers, but also reduce the potential pollution of the soil and ground water with leached fertilizers. Similarly, a synergistic action between beneficial microorganisms and their metabolites including trace plant hormones could improve soil conditions for plant development and directly affect the physiology of the plant, which could lead to increased branch and flowering, and eventually increasing fruit yield (Bulgari et al., 2019). Applications of IN-M1 may help tomato growers increasing fruit yield with reduced fertilizer usage following the ban of the fumigant methyl bromide which has led to a yield decline in Florida tomatoes (Guan et al., 2017).

Biostimulants may also help alleviate the damaging effect of salinity stress on plant growth (Bulgari et al., 2019). In a small-scale greenhouse trial with squash, weekly applications of IN-M1 significantly increased shoot fresh weight compared to the untreated control when squash plants were irrigated with salty water (Liu and Zhang, unpublished data). With increasing concerns over sea level rise and sea water intrusion, salinity stress may become a threat to vegetable production in south Florida including tomato. Application of IN-M1 could be a potential tool in mitigating salinity damage to vegetable crops in Florida.

In conclusion, our field trials suggested that the application of IN-M1 can stimulate plant growth and increase fruit yield of tomato in south Florida, particularly for extra-large and large sized fruit at early harvests. Further tests are needed to optimize its application in tomato and to investigate potential effects on mitigating salinity stress.

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Physiological, Growth and Yield Responses of Yellow Squash and Snapbean to a Potassium and Zinc Foliar Fertilizer

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Additional index words. leaf chlorophyll index, chlorophyll fluorescence, net CO_2 assimilation, transpiration, stomatal conductance

Yellow squash (*Cucurbita pepo* L.) and snapbean (*Phaseolus vulgaris* L.) are two of the main vegetable crops grown in south Florida. A field study was conducted to evaluate the effectiveness of a foliar-applied commercial fertilizer formulation (Strong Billow K®) containing potassium (K), zinc (Zn) and organic carbon on physiology, growth and yield of yellow squash and snapbean. Each crop was divided into three treatments: (T1) control (foliar applications of tap water), (T2) foliar applications of Strong Billow K® at the manufacturer's recommended rate (2.5 ml L^{-1}), and (T3) foliar application of K (as K_2O) and soluble Zn, applied at the same concentration as in the Strong Billow K® treatment. Application of Strong Billow K® had a positive effect on the leaf chlorophyll index (SPAD values), variable to maximum chlorophyll fluorescence ratio (Fv/Fm), net CO_2 assimilation (*A*), stomatal conductance (g_s), transpiration (*E*), and total fruit number per plant of squash and snapbean, as well as total fruit weight per plant of snapbean compared to the other two treatments. The effectiveness of Strong Billow K® on improving physiology, growth, and yield of yellow squash and snapbean were not due solely to the addition K and Zn in the Strong Billow K® formulation because applying these elements alone was no more effective than the control treatment.

The application of foliar fertilizers can decrease nitrogen (N), phosphorous (P), and potassium (K) soil fertilizers by up to 25%, thus reducing soil pollution and enhancing soil fertility (Alexander and Schoeder, 1987). Foliar fertilizers have been shown to increase net CO_2 assimilation (*A*), transpiration (*E*), stomatal conductance (g_s) (Haytova, 2015), vegetative and fruit biomass (Dimpka and Bindraban, 2016), and crop yield (Fageria et al., 2009). An advantage of foliar fertilizers over soil-applied formulations is that foliar fertilizers are often absorbed more rapidly than soil-applied fertilizers. Therefore, nutrients become available and accessible to the plant in a relatively short time (Baloch et al., 2008).

In addition to nutrient elements, commercial foliar fertilizer formulations often contain additional substances including vitamins, phytohormones, amino acids, humic acids, and adjuvants. Some of these substances provide additional benefits to the plant. For example, humic acids have been shown to increase soil water holding capacity (Behzad, 2014), plant dry weight, (Khaled and Fawy, 2011) and crop yields (Bakry et al., 2013).

Yellow squash (*Cucurbita pepo* L. cv. Enterprise) and snapbean (*Phaseolus vulgaris* L. cv. Caprice) also called green bean, are two of the main vegetable crops grown in Miami-Dade County, FL (Seal et al., 2016). Florida accounts for about 90% of the United States squash and fresh snapbean production. About 57% (11,050 ha) of Florida's snapbeans are grown in Miami-Dade County with a yield/ha of 321–494 bushels (13.6 kg = 1 bushel) (Zhang et. al., 2017). Miami-Dade grows about 2430 ha of yellow squash i and accounts for about 21% of Florida's yellow squash production (Hochmuth and Hanlon, 2017). The yield/ha is 741–1111 bushels (19.1 kg = 1 bushel) (Seal et. al., 2016). In southern Florida, yellow squash and snapbean are grown in oolitic limestone soils classified as Krome or Chekika very gravelly loam, characterized as loamy-skeletal, carbonatic hyperthermia lithic rendolls (Noble et al., 1996). These soils are alkaline (pH of 7.2–8.0), low in organic matter, very porous and have very low water- and nutrient-holding capacities (Li, 2001). Due to the high pH of these soils, minor elements applied to the soil are often bound or precipitated and not available to the plants unless applied as very expensive chelated formulations (Li, 2001). Application of foliar fertilizers, particularly minor elements, can overcome these soil nutrient limitations. Thus, identifying effective foliar micronutrient formulations is of paramount importance for vegetable crops grown on alkaline soils.

The objective of this study was to evaluate the effectiveness of a commercial foliar fertilizer formulation (Strong Billow K®; Grupo, Kaikun S.A.S., Medellin, Colombia) containing K_2O, zinc (Zn), and organic carbon on the physiology, growth, and yield of yellow squash and snapbean grown in an alkaline soil.

Materials and Methods

The study was conducted from 19 Dec. 2017 to 15 Feb. 2018 at the Tropical Research and Educational Center (UF-TREC) in Homestead, FL. (Lat. 25º30'40.809" N; Long. 80º30'3.983"). During the experiment, air temperature, humidity, rainfall and evapotranspiration data were collected from a Florida Automated

We would like to thank StrongBillow K®; Grupo, Kaikun S.A.S., Medelling, Colombia who supported this study. We thank our laboratory technical group and field workers who helped us maintain these crops.

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Weather Network (FAWN); <http://fawn.ifas.ufl.edu/> weather station located at UF-TREC. Evapotranspiration was calculated using the Blaney-Criddle method and the irrigation rate was calculated based on soil volumetric water content (Allen and Pruitt, 1986).

Yellow squash and snapbean were planted from seed on two raised beds, each 91.4 m long × 0.91 m wide × 0.18 m high with 1.83 m between centers. Prior to planting a 6–12–12 (N–P–K) granular fertilizer that contains 1.83% NO_2, 2.10% $CO(NH_2)_2$ soluble in water, 2.08% of $CO(NH_2)_2$ insoluble in water, 12% K_2O, 12% P_2O_5, and microelements including 0.25% Zn (Nutrient Agricultural Solutions, Homestead, FL) was soil-applied to each bed at 1307 kg·ha^{-1} in two furrows, each 0.20 m from and parallel to either side of the row and was incorporated 0.15 m into the top soil. Prior to covering the beds with plastic mulch, two polyethylene irrigation tubing (Ro-Drip, USA) with drip emitters spaced 0.3 m apart were placed 0.15 m apart on each side parallel to the center of each bed for irrigation. The beds were then covered with black polyethylene mulch (Kennco micro-combo, Kenco Manufacturing Co Inc., Atoka, OK). Holes (0.07 m diameter) were made manually in the plastic with a metallic hole digger 0.30 m apart within the bed. Two yellow squash seeds were planted in each hole in one-half of each bed and two snapbean seeds were planted in each hole on the other half of each bed. About one week after seeds germinated, the number of plants was thinned to one plant per hole. Beds were drip irrigated twice a day at 10:00 am and 4:00 pm, at a rate of 9.1 L per min/91.4 m for 15 min during the first two weeks, then the irrigation time was increased to 30 min until the end of the experiment. The plants were fertilized through the drip irrigation system twice a week for nine weeks with a 3–0–10 liquid fertilizer containing 2.99% NO_2, 0.01 % NH_3-H, and 10 % K_2O (Helena Chemical Company, Homestead, FL) at a rate of 178 L·ha^{-1} the first two weeks, 234 L·ha^{-1} for weeks three and four, and 297 L·ha^{-1} from week five through the end of the experiment.

Each crop received three treatments: (T1) control (foliar applications of tap water) applied to the point of run-off with a hand sprayer (Roundup sprayer, Fountainhead Group, Inc., New York, N.Y.) at the same time as applications of the other treatments, (T2) foliar applications of Strong Billow K® applied to the point of run off with a hand sprayer at the manufacturer's recommended rate (2.5 mL·L^{-1}) every 10 d beginning 7 d after germination, and (T3) foliar application of 35 g·L^{-1} of K_2O and 15 g·L^{-1} of Zn (the same concentration of K_2O and Zn as is in Strong Billow K® treatment) applied to the point of run off with a hand sprayer every 10 days beginning seven days after germination. The third treatment was included to determine if the effects of Strong Billow K® were due solely to the K_2O and Zn in the Strong Billow K® or to the complete Strong Billow K® formulation. For the first two applications, 5 mL of Strong Billow K® (T2) or K_2O and Zn solution (T3) were mixed with 2 L of water. For the remaining applications, 10 ml of Strong Billow K® (T2) or K_2O and Zn solution (T3) were mixed with 4 L of water. The pH of tap water, K_2O and Zn, and Strong Billow K® solutions were all between 7.3 and 7.5. Treatments for each crop were arranged in a randomized complete block design with five replications (15 blocks, each 3.05 m long with 10 plants) for yellow squash and four blocks (replications) (12 blocks, each 3.05 m long with 10 plants) for snapbean; the space between blocks was 0.61 m.

Net CO_2 assimilation, g_s, E, leaf chlorophyll fluorescence [the ratio of variable to maximum chlorophyll fluorescence (Fv/Fm)], and leaf chlorophyll index (SPAD values) were measured on the adaxial surface of one recently fully expanded mature leaf of 40 plants (8 plants within each of 5 blocks per treatment). There were eight plants in each block with the plants at the ends of each block considered buffer plants which were not measured). For yellow squash, A, g_s, and E were measured on three dates (11, 19, and 27 days after germination) with a CIRAS-3 portable gas exchange system (PP System Inc., Amesbury, MA). For A, g_s and E measurements, the photosynthetic photon flux density (PPFD) in the leaf cuvette, from a halogen light source, was set at 1000 μmol·m^{-2}·s^{-1} quanta, the reference CO_2 concentration in the cuvette was 410 μmol·mol^{-1} CO_2 and the air flow rate into the cuvette was 400 mL/min. The Fv/Fm, an indicator of damage to the photosynthetic apparatus (Krause and Weis, 1991), was measured with an OS-30 hand-held chlorophyll fluorimeter (Opti-Sciences Inc., Hudson, NH) on four dates (11, 29, 27, and 35 d after germination). The leaf chlorophyll index was measured with a SPAD-502 meter (Minolta, Inc., Osaka, Japan) on the same four dates that Fv/Fm was measured. For snapbeans, A, g_s and E were measured on three dates (12, 20, and 28 d after germination). The Fv/Fm and leaf chlorophyll index of snapbean were measured on four dates (12, 20, 28, and 36 d after germination).

Yellow squash fruits were harvested as they matured over a period of four weeks. Snapbeans were harvested 52 d after germination. Fruit number and fruit fresh weight were determined for eight plants in each block of each treatment and expressed as total fruit number and total fruit weight per plant.

Statistical differences among treatment means for fruit yields were determined by one-way analysis of variance (ANOVA) and a Waller-Duncan K-ratio test. Differences among treatments means for physiological variables were determined by repeated measures ANOVA. The SAS 9.4 statistical software package (SAS Institute, Cary, NC) was used for all statistical analyses.

Results

Throughout the experiment, the minimum and maximum temperatures were 4.2 °C and 28.5 °C, respectively, with minimum and maximum mean daily temperatures of 8.1 °C and 24.7 °C, respectively and an average daily temperature of 20.6 °C. During that period, mean daily relative humidity was 79.7%, mean daily rainfall was 0.58 mm, and mean daily evapotranspiration was 2.9 mm. Due to the soil properties, when the soil matrix potential reached between –10 to –30 kPa, the available soil water was 12.74 L/block. The irrigation rate was 9.08 L/block/day for the first two weeks and 18.2 L/block/day until the end of the experiment. For both crops, the mean difference between actual evapotranspiration and the irrigation rate per block was 0.005% in December, 0.007 % in January, and 0.02 % in February.

Yellow squash

The leaf chlorophyll index and Fv/Fm were significantly higher for plants in the Strong Billow K® treatment than for plants in the other two treatments (Fig. 1). There were no significant differences in A, E or g_s among any treatments on the first measurement date (Fig. 2). However, on the second measurement date, plants in the Strong Billow K® treatment had higher A and g_s than plants in the other two treatments and higher E than plants in the control treatment (Fig. 2). On the third measurement date, there were no differences in A among treatments. However, on that date, E was higher for plants in the Strong Billow K® treatment than in the other treatments, and g_s was higher for plants in the Strong Billow K® treatment compared to the control treatment (Fig. 2).

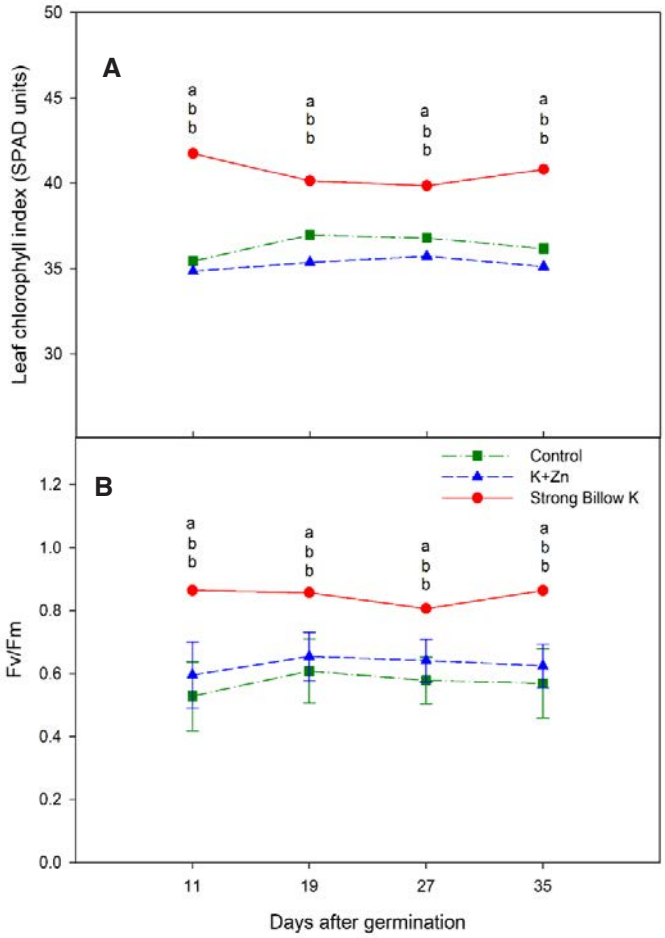

Fig. 1. Effects of foliar fertilizer treatments on **A**) leaf chlorophyll index and **B**) ratio of variable to maximum chlorophyll fluorescence (Fv/Fm) of yellow squash. Different letters indicate significant difference among treatment means ($P < 0.05$). Symbols represent means of 8 plants per 5 blocks (replicates) and bars indicate + 1 standard error.

Table 1. Effect of foliar fertilizer treatment on total leaf dry weight, total fruit number, and total fruit weight per yellow squash plant.

Treatment	Fruit number/plant	Total fresh fruit wt/plant (kg)
Strong Billow	13.4 a	4.4a
Control	8.4 b	2.5a
K and Zn	7.6 b	2.4a

[z]Different letters within columns indicate a significant difference among treatment means according to the Waller-Duncan K ratio test ($P < 0.05$).

Total fruit number per plant was significantly higher in the Strong Billow K® treatment than in the other two treatments. The yield per hectare was higher than commercials yields, it was 29.15 kg/0.0014 ha or 0.20 kg/plant. Although not statistically significant ($P > 0.05$), the total fresh fruit weight per plant was more than 30% higher in the Strong Billow K® treatment than in the K_2O and Zn or control treatments (Table 1).

Snapbean

On all three measurement dates, the leaf chlorophyll index was significantly higher for plants in the Strong Billow K® treatment than plants in the other two treatments (Fig. 3). The Fv/Fm was significantly higher for plants in the Strong Billow K® treatment than plants in the other two treatments on the second and last measurement dates (Fig. 3). On the first and third measurement dates, Fv/Fm was significantly higher for plants in the Strong billow K® treatment than those in the control treatment, but there

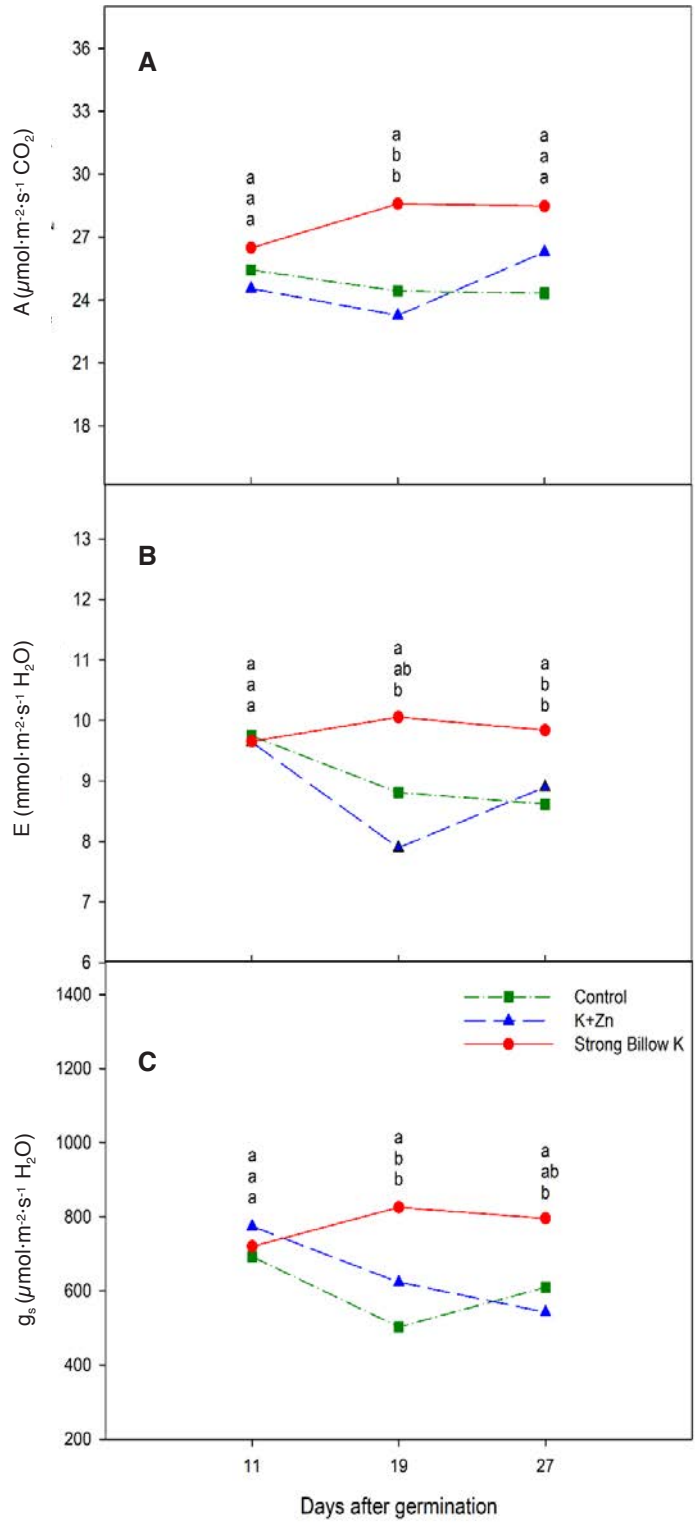

Fig. 2. Effect of foliar fertilizer treatments on **A**) net CO_2 assimilation (A), **B**) stomatal conductance (g_s), and **C**) transpiration (E) of yellow squash. Different letters indicate significant difference among treatment means ($P < 0.05$). Symbols represent means of 8 plants per each of 5 blocks (replicates) and bars indicated + 1 standard error.

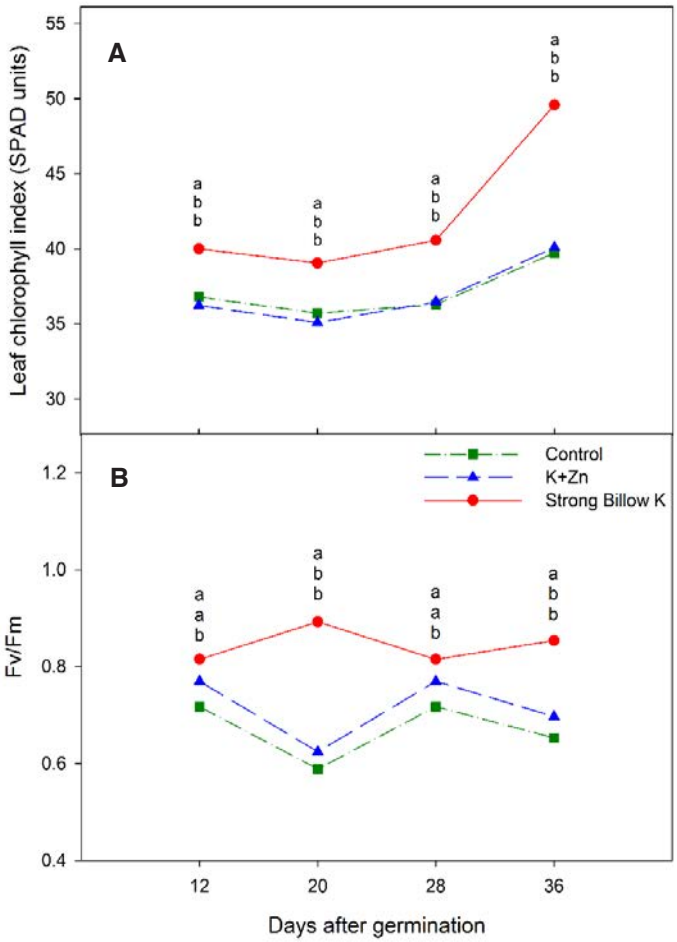

Fig. 3. Effects of foliar fertilizer treatments on **A**) leaf chlorophyll index and **B**) ratio of variable to maximum chlorophyll fluorescence (Fv/Fm) of snapbeans. Different letters indicate significant difference among treatment means ($P < 0.05$). Symbols represent means of 8 plants per each of 4 blocks (replicates) and bars indicate + 1 standard error.

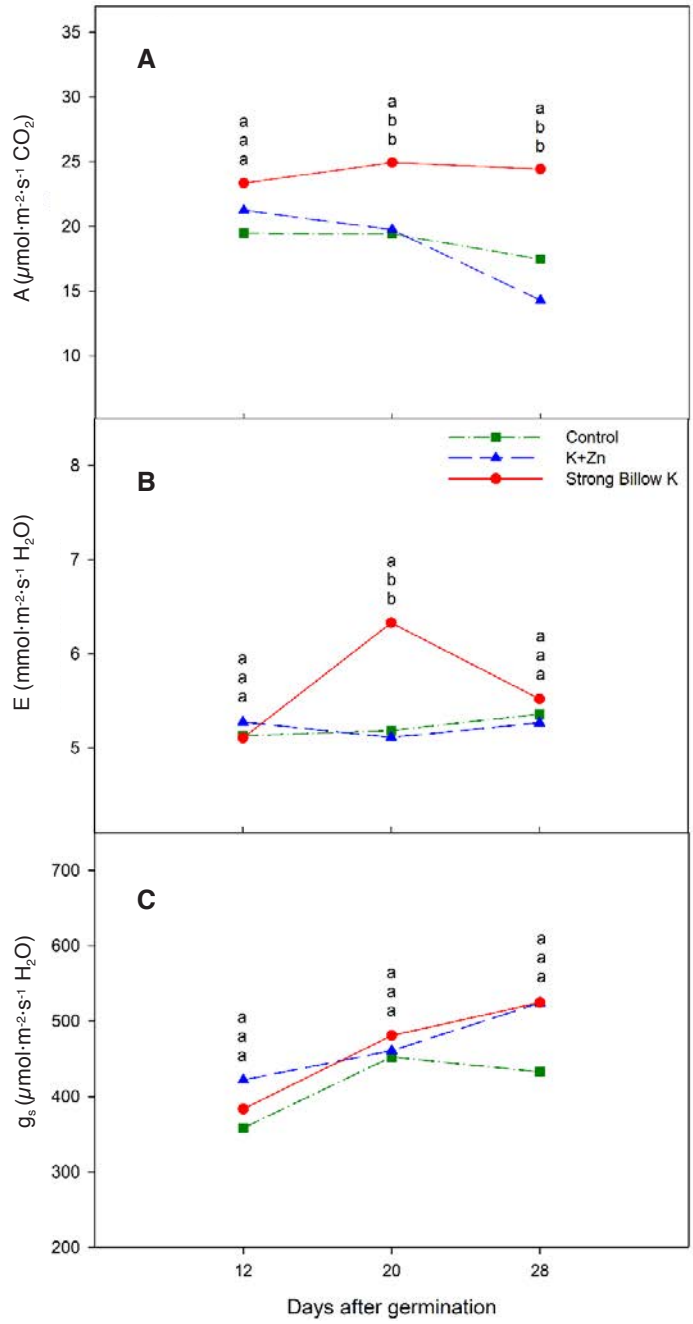

Fig. 4. Effect of foliar fertilizer treatments on **A**) net CO_2 assimilation (A), **B**) stomatal conductance (g_s), and **C**) transpiration (E) of snapbeans. Different letters indicate significant differences among treatment means ($P < 0.05$). Symbols represent means of 8 plants per each of 4 blocks (replicates) and bars indicate + 1 standard error.

was no significant difference in Fv/Fm between the Strong Billow K® treatment and the K_2O and Zn treatment on those measurement dates (Fig. 3).

Net CO_2 assimilation was significantly higher for plants in the Strong billow K® treatment than plant in the other treatments on the second and third measurement dates (Fig. 4). Transpiration was significantly higher for plants in the Strong Billow K® treatment than for plants in the other treatments only on the second measurement date, whereas there were no significant differences in E among treatments on the other measurement dates (Fig. 4). There were no significant differences in g_s among treatments (Fig. 4).

Total fruit number per plant and total fresh fruit weight per plant were significantly higher in the Strong Billow K® treatment than in the other two treatments (Table 2).

Discussion

The positive effects of Strong Billow K® foliar fertilizer on the physiology and yield of squash and snapbean in this study were probably not just a result of the addition of K_2O and Zn in the fertilizer formulation because foliar application of K_2O and Zn alone did not have the same positive effects. Thus, another component of Strong Billow K®, perhaps organic carbon, may

Table 2. Effect of foliar fertilizer treatment on total leaf dry weight, total fruit number, and total fruit weight per snapbean plant.

Treatment	Fruit number/plant	Total fresh fruit wt/plant (kg)
Strong Billow	50.3 a	1.88 a
Control	37.6 b	1.04 b
K and Zn	30.6 b	1.53 b

[z]Different letters within columns indicate a significant difference among treatment means according to a Waller-Duncan K ratio test ($P < 0.05$).

have either had a direct effect on plant physiology and growth, or indirectly affected K_2O or Zn in the solution, making it more easily absorbed or metabolized by the plants. Many studies have shown positive results using similar combinations of K_2O and Zn with other substances. For example, K combined with humic acid can facilitate the delivery of high levels of organic soluble K to the plant through foliar or soil applications (Shafeek et al., 2015). In a previous study, the combination of foliar Zn and chitosan had a positive effect on seed yield of dry bean (Ibrahim and Ramadan, 2015). In the present experiment, foliar application of Strong Billow K® on yellow squash and snapbean resulted in an increase in the leaf chlorophyll index and Fv/Fm in both yellow squash and snapbean. Kaya et al. (2002) observed an increase in chlorophyll content in tomato leaves after foliar application of Zn at 0.35 $\mu mol \cdot L^{-1}$ compared with a control treatment with 7.7 $\mu mol \cdot L^{-1}$ Zn in the nutrient solution. Similar results were found in several varieties of mungbean with 2 µM of Zn applied to the foliage (Tayyeba et al., 2013). An increase in the leaf chlorophyll index was observed in green bean, cucumber, and tomato with foliar applications of aminochelate (Souri et al., 2017), which may have chelated Zn to a form more available for plant metabolism. Brennan (1991) showed that foliar application of Zn in a chelated form (ZnEDTA) increased grain yield of wheat compared to foliar application of a non-chelated form of Zn ($ZnSO_4$). The higher Fv/Fm of yellow squash and snapbean the Strong Billow K® treatment compared to the other treatments studied may have also been due, at least in part, to K_2O in the Strong Billow K®. In coriander plants, increased leaf chlorophyll index and Fv/Fm were observed with foliar applications of K (Roosta, 2014).

In both yellow squash and snapbean, foliar applications of Strong Billow K® resulted in higher *A* and *E* compared to foliar application of K_2O and Zn or tap water. Similarly, an increase in leaf gas exchange variables of zucchini was observed with the foliar application of three commercial products with different chemical formulations [Fitona® 3 (5.20% K_2O and 0.007% Zn), Hortigrow® (20% K_2O and 0.02% Zn), and Humustim® (7.83% K_2O and 0.01% Zn)], each containing K and Zn (Haytova, 2015). An increase in *A* and *E* were observed on jatropha plants with the foliar application of BAM-FXTM, containing 7.14% Zn, when the soil application rate of nitrogen (N), phosphorous (P) and potassium (K) were low (Bosco de Oliveira et al., 2019). In coffee plants, *A* and *gs* increased 55% in response to a foliar application of $ZnSO_4$, N and P compared to a control treatment with no foliar fertilizer applied (Rossi et al., 2018).

Fruit number per plant of both yellow squash and snapbean was significantly higher for plants treated with Strong Billow K® than those in the other two treatments. Foliar application of K or Zn has been shown to increase crop reproductive development and yield. For example, the number of seeds and seed weight per plant increased with the combination of Zn and humic acid applied to the foliage of bean plants (Kaya et al., 2005). Also, foliar application of Zn, K, or Mg had a positive effect on yield and growth of several varieties of mungbean (Thalooth et al., 2006) and foliar application of 100 or 200 ppm K increased plant growth and yield of pepper (Hussein et al., 2012). Kaya and Higgs (2002) reported an increase in chlorophyll content, dry weight and fruit yield of tomato with foliar applications of Zn. In contrast to these findings, in the present study, increased yield of yellow squash and growth and yield of snapbean receiving foliar applications of Strong Billow K® was apparently not solely due to K_2O and/or Zn in the formulation, even though leaf Zn concentration (results not shown) was higher in the Strong Billow K® treatment than in the control treatment for both crops, because applying those elements alone was no more effective than applying tap water. Therefore, the positive effects of Strong Billow K® on snapbean growth and squash and snapbean yields may have been due to direct effects of other components in the Strong Billow K® formulation on plant growth and yield or indirect effects of other components in the formulation on Zn and or K absorption through the foliage. Nevertheless, foliar applications Strong Billow K® significantly increased growth of snapbeans and yield of squash and snapbeans.

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Management of the Pepper Weevil, *Anthonomus eugenii* Cano (Coleoptera: Curculionidae) using Bio-rational Insecticides In Combination with Aggregation Pheromone

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Pepper, *Capsicum annum* L., is an important crop grown mostly in tropical and subtropical regions, including Florida and several other states in the southern region of the United States. The pepper weevil, Anthonomus eugenii, is the most harmful insect pest of pepper in Florida and other tropical and subtropical regions of North, Central, and South America. All commercially cultivated peppers are susceptible to pepper weevil including Jalapeño pepper. The use of broad-spectrum insecticides is the major management tool used for pepper weevil control; their continuous use has led to the development of resistance. Therefore, alternative approaches are needed for effective control. We conducted studies evaluating the effect of bio-rational and conventional insecticides in combination with an aggregation pheromone against pepper weevil under field conditions. Parameters evaluated include the number of adults on plants, the number of infested fallen fruits, and marketable yield. Infested fruits collected from each treatment plot were dissected to evaluate larval, pupal, and adult density. Results show that the number of adults observed on plants and the number of infested fruits did not differ statistically when the same insecticide treatment was compared with and without the pheromone. However, there were statistical differences when treatments were compared across all sampling dates. Marketable yield was lower in the pheromone treated plots compared to plots without the pheromone. This information provides an insight into developing a sustainable approach to manage pepper weevil.

Pepper production is greatly challenged by pests including thrips, aphids, whiteflies, root-knot nematodes, red spider mites, fruit borers (Dagnoko et al., 2013), and the most economically important pest in the southern US, *Anthonomus eugenii* Cano commonly referred to as the pepper weevil (Andrews et al., 1986). Various insecticides used to manage pepper weevil have diverse modes of action and may negatively affect natural enemy populations and other associated organisms (Naylor and Ehrlich, 1997).

The major management tool used for the control of pepper weevil is conventional insecticides, but even with these insecticides, control is difficult, especially when the pepper weevil population is high (Seal and Lamberts, 2012; Addesso et al., 2014). Actara® (Thiamethoxam, Syngenta Crop Protection, Inc.) and Vydate® (Oxamyl, DuPont) are standard insecticides used by growers alone or in rotation to manage the pepper weevil population and increase yield (Addesso et al., 2014). The pepper weevil population's susceptibility to these insecticides depends on the history and type of insecticides used in each location. Servin-Villegas et al. (2008) reported that pepper weevil adults were more susceptible to some groups of insecticides at one location compared to the same insecticides at another location. Alternative management strategies are being sought to reduce the sole dependence on broad-spectrum insecticides.

Growers apply broad-spectrum insecticides 2–3 times per week to manage pepper weevil by targeting the adults while immature stages, being hidden inside fruits, escape insecticides. Thus, the field remains infested due to the continuous emergence of new adults and by incoming adult populations from alternate hosts. Repeated use of broad-spectrum insecticides enhances the development of resistance in pepper weevil and eliminates pepper weevil's natural enemies (Servin-Villegas et al., 2008).

To reduce the sole dependence on broad-spectrum insecticides and their effects on natural enemies and reduce the selection pressure for the development of resistance, it is important to integrate other management strategies, including bio-rational insecticides. However, there are few studies where bio-rational insecticides have been used for the control of pepper weevil and have been found to be effective against other weevils in different genera (Addesso et al., 2014; Reddy and Antwi, 2016).

In a laboratory study, Reddy et al. (2016) found that bio-rational insecticides, including *Beauveria bassiana*, Azadirachtin, *Metarhizium brunneum*, *B. bassiana* + pyrethrins, *B. bassiana* +

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cold-pressed neem extract, and Spinosad were effective against the larvae of alfalfa weevil. The same insecticides were tested against the wheat head armyworm, *Dargida diffusa* (Lepidoptera: Noctuidae) and Entrust® was the most effective, causing a 100% mortality within three days of treatment, followed by Xpectro® (Reddy and Antwi 2016).

Many of these reduced-risk insecticides are effective against the larvae, the major damage causing life-stage of insect pests, as seen above in the alfalfa weevil. For pepper weevil, all immature life stages develop within the fruit, making the adult the only life stage exposed to insecticides, thus making management difficult. For this reason, most of the products, including currently available insecticides, are directed at the control of the adult stages of the pepper weevil. Therefore, the goal is to develop management strategies to keep the pepper weevil population below the economic threshold level and introduce bio-rational tactics to reduce selection pressure from conventional pesticides.

For weevil species, an aggregation pheromone is produced by males and is used to attract both sexes (Tewari et al., 2014). An aggregation pheromone has been isolated and identified for pepper weevil, and its field activity was also evaluated. However, Eller et al. (1994) said there was a need to improve the efficiency of the traps and the attractiveness of the aggregation pheromone formulation to the pepper weevil. The use of pheromones combined with other management tactics has been deemed a potential strategy in reducing the use of broad-spectrum insecticides and the risk of their deleterious effects on non-target and beneficial insects (Witzgall et al., 2008; Eller and Palmquist, 2014).

Since no research has documented the effects of some of the newer bio-rational insecticides for pepper weevil management, this research tested the efficacy of bio-rational insecticides in combination with aggregation pheromones.

Materials and Methods

Study site and area. The study was carried out in research plots at the Tropical Research and Education Center (TREC) in Homestead, Fla. (25.513°N; –80.504°W) and 3 (25.511°N; –80.501°W). The soil type is a Krome gravelly loam soil classified as a loamy-skeletal, carbonatic hyperthermia lithic rendoll, which consists of 67% limestone pebbles (>2 mm) and 33% finer particles (Noble et al., 1996).

Field preparation. The soil was prepared by plowing the field with a moldboard plow (CASE IH agriculture), and then using a disking machine (Athens Plow Co Inc., TN, USA). Beds were raised to a height of 15 cm with a width of 91 cm and 1.83 m between centers using Kennco superbedders (Kennco Manufacturing Co. Inc., Atoka, OK, USA). Granular fertilizer (6 N–12 P–12 K) at the rate of 1344 kg/ha (1200 lb/acre) was applied at planting as a broadcast and incorporated before covering the beds with plastic mulch (manufactured by Canslit Inc. Victoriaville, Quebec, Canada, and supplied by IMAFLEX USA, Inc.). The polyethylene mulch was placed on the beds using a plastic layer (Kennco micro-combo, Kennco Manufacturing Co. Inc., Atoka, OK). When laying down plastic, two drip tubes (RO-Drip) with emitters spaced 30 cm apart, one on either side of the plant row at a spacing of 15 cm, were placed for irrigation. 'Jalapeño' transplants were planted at the center of each bed in between the two drip tubes. Planting, holes (7 cm diameter) were made manually using a metallic hole digger, maintaining 31 cm (12 inches) between transplants.

Plant material. 'Jalapeño' pepper transplants, variety PS 11435807, were provided by Mobley Plant World LLC, Labelle, FL. Transplants were received six-week after germination.

Experimental design and treatments. A randomized complete block design was used to evaluate insecticide and pheromone treatments. The plots consisted of treatments with and without pheromone traps. Insecticide treatments included Actara® (with and without the pheromone), Xpectro® (with and without the pheromone), and untreated controls (with and without pheromones). All treatments were randomized within blocks and replicated three times. Each treatment plot was 4.6 m (15 ft) long with a 3.05 (10 ft) non-planted buffer area that separated treatments within the block. For the treatments with the pheromone, a yellow sticky trap (15 cm × 30 cm) baited with the pepper weevil aggregation pheromone septum was tied on an iron rod (122 cm high and 10 cm diameter) and placed in the center of each treatment plot at the same height as the canopy of the plants. Traps were placed the same day jalapeño peppers were transplanted and were checked weekly for trapped pepper weevil adults. Traps were renewed every two weeks with new traps and maintained to observe their effect on the pepper weevil population throughout the season. During the insecticide application, the pheromone traps were covered with polythene bags to prevent spray drift.

Crop management. Pepper plants were subjected to recommended cultural practices (as described in Boyd et al., 2018) which include irrigation, weeding, fertilizer applications, etc. throughout the season. After planting, plots were subjected to close observation, scouting for the presence and infestation of pepper weevil. Plants were irrigated twice a day (10:00 am and 4:00 pm) for half an hour using the drip irrigation system already described. Immediately after transplanting, the base of each transplant was drenched with starter fertilizer solution (20 N–20 P–20 K) (0.75 oz/gal of water) (Diamond R Fertilizer Inc. Ft. Pierce, Fla) using a back-pack sprayer without a nozzle tip. Granular fertilizer (6 N–6 P–6 K) (Loveland Products Inc., Greely, CO) was applied every three weeks after planting. The fertilizer was applied 20 cm from and parallel to the side of the transplants or plants and incorporated within the top 15 cm of the soil. Weeding was done manually when weeds were present. Bravo Weather Stik® (Chlorothalonil, IRAC group M5 fungicide, Syngenta Crop Protection Inc., Greensboro, NC) was applied at 1.75 L/ha to reduce the effects of fungal pathogens.

Application and evaluation of insecticide treatments. The application of insecticide treatments was initiated five to six weeks after planting and continued at 7 d intervals. Insecticides (Table 1.) were applied to the foliage using a hand-propelled backpack sprayer (Birchmeier 15.14-L Backpack Sprayer, model IRIS, Stetten Switzerland) delivering 280 l/ha (30 GPA) at 2.11 kg/cm2 (30 psi). Insecticides were sprayed weekly, eight and six times in the first and second studies, respectively. The first application in the first study was on 27 Dec. 2019 and that of the second study was on 18 Feb. 2020.

Sampling. Evaluation of insecticide treatments was made by thoroughly checking five randomly selected plants for pepper weevil adults 24 h after each spray application between 10 am and 12 pm. On the same day, fallen infested fruits from randomly selected five plants/treatment plot were collected. These were transported to the Vegetable IPM laboratory, TREC where they were dissected with a dissecting scalpel, and pepper weevils at different life stages (larva, pupa, and adult) were counted and

Table 1. Insecticides and application rates used for field studies.

Chemical name	Trade name	Application rates kg/ha	IRAC Group	Company
Beauveria bassiana GHA + refined pyrethrum extract	Xpectro® OD	2.24	UNF	LAM International
Heat killed *Burkholderia* spp. Strain A396	Venerate® XC	6.73	UNB	Marrone Bio Innovations
GS-omega/kappa-Hxtx-Hv1a	Spear® -T	4.48	32	Vestaron
Esfenvalerate	Asana®	0.56	3A	Valent
Thiamethoxam	Actara®	0.28	4A	Syngenta
Oxamyl	Vydate®	3.36	1A	Dupont
Untreated control	–	–		–

recorded. Means across all sampling dates (MASD) were obtained by pooling all the data on all sampling dates. At harvest, marketable fruits from each plot were counted and weighed a using 31.75 kg (70 lb) capacity scale (CCI Scale Company, Ventura, CA).

Statistical analysis. Response variables measured were adults on plants, infested fruit counts, and the number of pepper weevil larvae, pupae, and adults in dissected fruits. Data were subjected to a square-root transformation (x^2) before statistical analysis to meet the assumption of normality and homogeneity of variance. The non-transformed means were reported in the tables. We analyzed the data using a repeated measures linear mixed model to determine the fixed effect of treatments, sampling dates and their interaction on the response variables. An auto regressive correlation structure was used to account for the repeated measures over time. Kenward-Roger's method was used to estimate degrees of freedom. (PROC GLIMMIX model, SAS version 9.4, SAS Institute, Inc. Cary, NC, 2013). For the response variables in each treatment and on each sampling dates, mean differences among treatments, (least square means) were separated using Tukey's multiple comparisons, and in all cases the level of statistical significance was set to $\alpha = 0.05$. A process called slicing was used to simplify comparisons when an interaction was significant.

Results

The abundance of pepper weevil adults on five randomly selected jalapeño pepper plants per treatment plot. In the Nov. 2019 study, the mean number of adults observed on plants in the untreated control without the pheromone was significantly higher than the rest of the treatments on the last sampling date ($F_{5, 158} = 7.30$; $P = 0.0001$). When means were compared across all sampling dates, adults were significantly lower in the Xpectro® treatment with and without the pheromone, the Actara® treatment without the pheromone and the control with the pheromone compared to Actara® with the pheromone and the untreated control without the pheromone (Fig. 1). When this experiment was repeated in Dec. 2019, the mean number of adults across all sampling dates in the Actara® without pheromone treatment was significantly lower compared to Xpectro® with the pheromone, and the untreated control with and without pheromone (Fig. 2).

Infested fallen fruits collected from 5 randomly selected plants per treatment plot. Infested fruits were absent on the first three sampling dates in the Nov. 2019 study. When means were compared across all sampling dates, Xpectro® with the pheromone and Actara® without the pheromone treatments had statistically lower mean numbers of infested fruits compared to the untreated control treatment with and without the pheromone (Fig. 3).

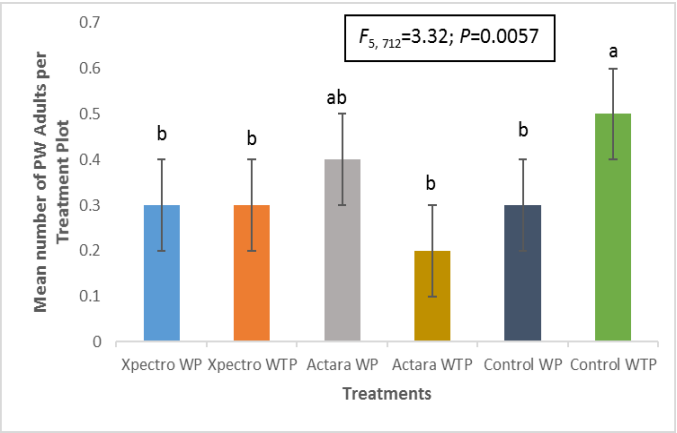

Fig. 1. Mean ± SE number of pepper weevil adults observed across all sampling dates on five randomly selected jalapeño plants per treatment plot (November 2019). Means with the same letter are not significantly different at P≤ 0.05 according to Tukey's HSD test. Xpectro® insecticide with pheromone (X-WP), Xpectro® insecticide without pheromone (X-WTP), Actara® insecticide with pheromone (A-WP), Actara® insecticide without pheromone (A-WTP), untreated control with pheromone (C-WP), untreated control without pheromone (C-WTP).

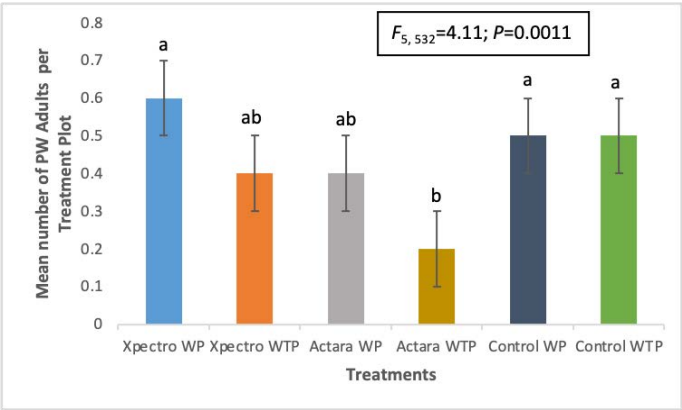

Fig. 2. Mean ± SE number of pepper weevil adults observed across all sampling dates on five randomly selected jalapeño plants per treatment plot (Dec. 2019). Means with the same letter are not significantly different at $P \leq 0.05$ according to Tukey's HSD test. Xpectro® insecticide with pheromone (X-WP), Xpectro® insecticide without pheromone (X-WTP), Actara® insecticide with pheromone (A-WP), Actara® insecticide without pheromone (A-WTP), untreated control with pheromone (C-WP), untreated control without pheromone (C-WTP).

In the Dec. 2019 study, the Xpectro® treatments and Actara® without the pheromone treatment had significantly lower mean numbers of infested fruits compared to the untreated control treatments (Fig. 4).

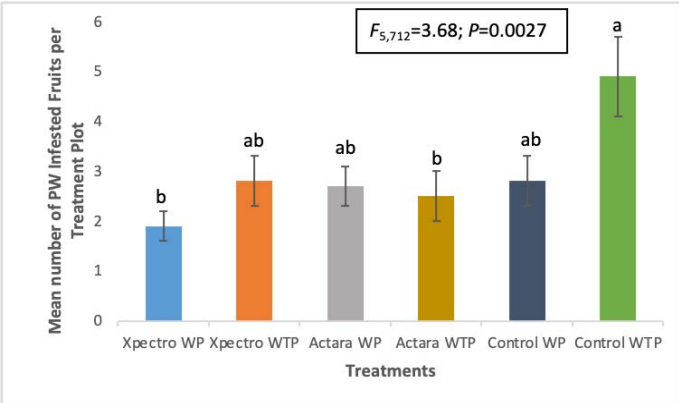

Fig. 3. Mean ± SE number of pepper weevil infested fruits collected from five randomly selected jalapeño plants per treatment plot (Nov. 2019). Means with the same letter are not significantly different at $P \leq 0.05$ according to Tukey's HSD test. Xpectro® insecticide with pheromone (X-WP), Xpectro® insecticide without pheromone (X-WTP), Actara® insecticide with pheromone (A-WP), Actara® insecticide without pheromone (A-WTP), untreated control with pheromone (C-WP), untreated control without pheromone (C-WTP).

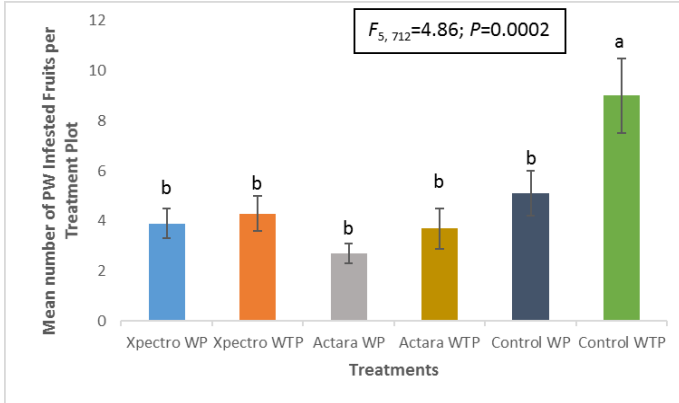

Fig. 5. Mean ± SE number of pepper weevil (larvae+pupae+adult) recorded from infested fruits in the 2020 studies (Nov. 2019). Means with the same letter are not significantly different at $P \leq 0.05$ according to Tukey's HSD test. Xpectro® insecticide with pheromone (X-WP), Xpectro® insecticide without pheromone (X-WTP), Actara® insecticide with pheromone (A-WP), Actara® insecticide without pheromone (A-WTP), untreated control with pheromone (C-WP), untreated control without pheromone (C-WTP).

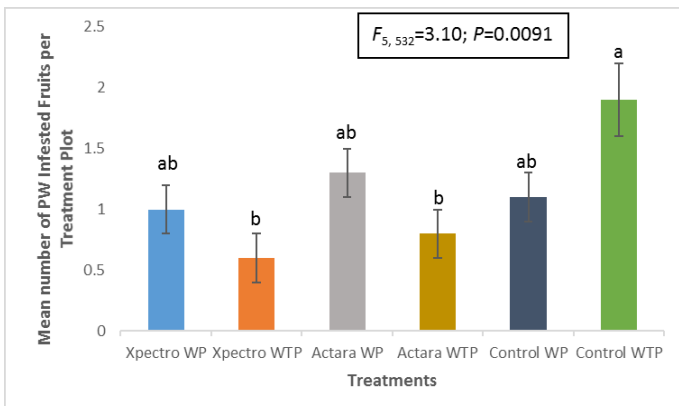

Fig. 4. Mean across all sampling dates ± SE number of pepper weevil infested fruits collected from five randomly selected jalapeño plants per treatment plot (Dec. 2019). Means with the same letter are not significantly different at $P \leq 0.05$ according to Tukey's HSD test. Xpectro® insecticide with pheromone (X-WP), Xpectro® insecticide without pheromone (X-WTP), Actara® insecticide with pheromone (A-WP), Actara® insecticide without pheromone (A-WTP), untreated control with pheromone (C-WP), untreated control without pheromone (C-WTP).

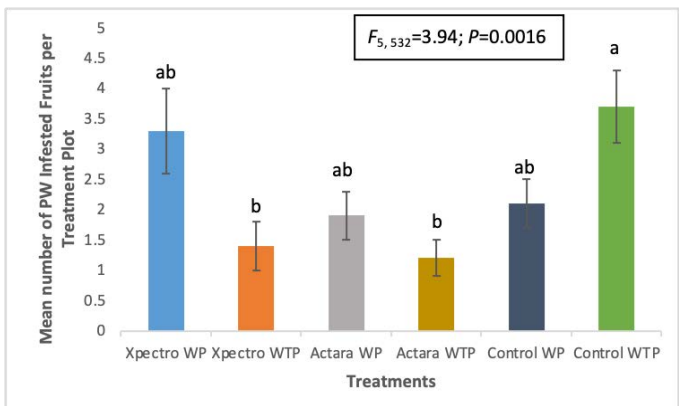

Fig. 6. Mean ± SE number of pepper weevil (larvae+pupae+adult) recorded from infested fruits in the 2020 studies (Dec. 2019). Means with by the same letter are not significantly different at $P \leq 0.05$ according to Tukey's HSD test. Xpectro® insecticide with pheromone (X-WP), Xpectro® insecticide without pheromone (X-WTP), Actara® insecticide with pheromone (A-WP), Actara® insecticide without pheromone (A-WTP), untreated control with pheromone (C-WP), untreated control without pheromone (C-WTP).

Mean number of pepper weevil (larvae, pupae, and adult) in infested fruits. In the Nov. 2019 study, when the mean number of pepper weevil (larvae+pupae+adults) was considered, the untreated control plot without the pheromone had a significantly higher number of pepper weevils compared to the rest of the treatments (Fig. 5). In the Dec. 2019 study, the Actara® and Xpectro® treatments without the pheromone had significantly lower pepper weevil density in infested fruits compared to the untreated control and the rest of the treatments (Fig. 6).

Marketable yield at harvest. The mean marketable yield at harvest in the treatments without the pheromone was higher than the treatments with the pheromones in the Nov. 2019 study (Table 2). When means were compared across pheromone treated plots, Actara® and Xpectro® treatments had higher fruit yield compared to the untreated control, although, there were no statistical differences in yield among the treatments. A similar trend was observed in the plots without the pheromone in the Nov. 2019 study.

Table 2. Mean ± SE marketable yield at harvest (kg/ha) in the presence and absence of pheromone in 2020.

Treatment	Nov. 2019 Study	Dec. 2019 Study
X-WP	8753 ± 6246 a	0.0 ± 0.0 b
X-WTP	17124 ± 14686 a	699 ± 314 b
A-WP	15222 ± 1087 a	8037 ± 4006 a
A-WTP	18320 ± 7243 a	11688 ± 2322 a
C-WP	5436 ± 2677 a	0.0 ± 0.0 b
C-WTP	13700 ± 5481 a	959 ± 384 b
	F = 1.12; P = 0.4074	F=15.73; P < 0.0001

Means within the same column followed by the same letter are not significantly different at $P \leq 0.05$ according to Tukey's HSD test. Xpectro® insecticide with pheromone (X-WP), Actara® insecticide with pheromone (A-WP), untreated control with pheromone (C-WP).

No marketable fruits were harvested from the Xpectro® and the untreated control plots with the pheromone in the Dec. 2019 study (Table 2). Actara® treated plots, with and without the pheromone had significantly higher fruit yield compared with the Xpectro® and untreated control treatments, with and without pheromone. Marketable yield among treatments did not differ statistically ($F_{5, 10}$ = 1.12; P = 0.4074) in the Nov. 2019 study but differed statistically in the Dec. 2019 study ($F_{5, 12}$ = 15.73; P < 0.0001).

Adults trapped on the pherocon traps. On the first sampling date, no pepper weevil adults were trapped by the pheromone traps in the Nov. 2019 study. For the rest of the sampling dates, pepper weevil adults were trapped, and there were no significant differences in the number of adults attracted and trapped by the pheromone. Adults were trapped by the pheromone traps on all sampling dates in the second study, although, they did not differ statistically (Table 3). The first-time adults were observed in the treatments correlated with the time adults were observed on the pheromone traps in the first study. Adults were absent on the first sampling dates both on the pheromone traps and on the plants.

Table 3. Means ± SE number of pepper weevil adults recorded on the pheromone traps in the treatments with pheromone (Nov. 2019 and Dec. 2019).

SW[z] (dates)	X-WP	A-WP	C-WP	Statistics (df = 5) F; P
Nov. 2019 Study				
1 (12–28)	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.00; 0.00
2 (1–03)	0.7 ± 0.7 a	1.3 ± 0.7 a	0.7 ± 0.3 a	0.34; 0.7220
3 (1–10)	0.7 ± 0.3 a	1.7 ± 1.7 a	3.3 ± 0.9 a	1.69; 0.2612
4 (1–18)	0.3 ± 0.3 a	0.7 ± 0.7 a	1.0 ± 0.6 a	0.36; 0.7143
5 (1–24)	0.7 ± 0.3 a	1.0 ± 0.6 a	0.3 ± 0.3 a	0.50; 0.6297
6 (1–30)	2.7 ± 2.2 a	0.7 ± 0.3 a	2.0 ± 0.6 a	0.58; 0.5869
7 (2–07)	10.0 ± 1.5 a	4.3 ± 1.2 a	6.0 ± 3.1 a	1.93; 0.2251
8 (2–15)	8.7 ± 1.5 a	3.0 ± 1.2 b	2.7 ± 0.7 b	7.38; 0.0024
9 (2–20)	7.3 ± 1.2 a	5.7 ± 0.9 a	4.7 ± 0.7 a	1.96; 0.2212
Dec. 2019 Study				
1 (2–19)	4.0 ± 0.6 a	2.0 ± 1.0 a	1.3 ± 0.3 a	3.96; 0.0801
2 (2–25)	2.7 ± 0.7 a	0.7 ± 0.7 a	0.3 ± 0.3 a	4.53; 0.0633
3 (2–30)	1.7 ± 0.3 a	0.3 ± 0.3 a	1.3 ± 0.3 a	5.02; 0.0524
4 (3–04)	1.0 ± 0.6 a	3.3 ± 1.8 a	1.7 ± 1.2 a	0.51; 0.6267
5 (3–12)	6.3 ± 3.3 a	4.7 ± 1.9 a	4.7 ± 2.2 a	0.11; 0.8966
6 (3–21)	5.3 ± 0.9 a	2.0 ± 1.0 a	3.3 ± 1.8 a	1.27; 0.3472

Means within the same row followed by the same letter are not significantly different at $P \leq 0.05$ according to Tukey's HSD test. Sampling weeks (SW[z]). Xpectro® insecticide with pheromone (X-WP), Xpectro® insecticide without pheromone (X-WTP), Actara® insecticide with pheromone (A-WP), Actara® insecticide without pheromone (A-WTP), untreated control with pheromone (C-WP), untreated control without pheromone (C-WTP).

Discussion

Results obtained from these trials provided additional information to previous studies. In our studies, in the treatments using pheromone traps, adults were found on the pheromone traps the same day that they were observed on plants. We could not completely count on finding adults on the pheromone traps before the insecticide application was made. To have good results, Addesso et al. (2011) recommended a combination of pheromone lures and host plant volatiles to attract males and virgin females. In our studies, pepper weevils attracted to the traps by the pheromone were not sexed, but it is possible that only males and virgin females were attracted while the gravid females sought oviposition sites instead of being attracted by the pheromone lures. The banana root weevil (*Cosmopolites sordidus*) response was enhanced when host plant odors, such as fermented banana pseudostem tissue, were added to the synthetic aggregation pheromone Cosmolure+® (Tinzaara et al. 2007). Andrews et al. (1986), mentioned that an action threshold can be established if there is a strong correlation between adults captured on pheromone traps and the abundance and damage of pepper weevil.

Pepper plants treated with Actara® reduced the number of pepper weevil adults found on plants, the number of infested fruits when compared to the untreated control. However, they provided some reduction in infestation in terms of the number of infested fruits.

Effectiveness of the insecticides were not clear enough to determine the most effective one. However, Actara®, a standard insecticide used by growers proved to be effective in some cases. This was evident in the Actara® treated plots without the pheromone, which performed better in terms of marketable yield and lower numbers of pepper weevil adults compared to the rest of the treatments in both TREC and in off-station studies.

Controlled laboratory studies are needed to evaluate the mortality of pepper weevil adults in response to all the insecticides evaluated in the field.

The effect of the presence or absence of the pheromone was unpredictable in this study, although marketable yield was higher in treatment plots without the pheromone compared to treatment plots with the pheromone. For future studies, yellow sticky traps without pheromone lures could be placed in plots where no pheromone was used. This will help observe the differences between the number of trapped adults between pheromone treated plots and plots with only the yellow sticky traps. We observed that pepper weevils were attracted by the pheromone lures, but it is possible that yellow sticky traps were not efficient in trapping the attracted weevils, therefore, a more effective trapping system may need to be established for better results.

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Harvest Yields for Sweet Corn Variety Trial In Northeast Florida

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Sweet corn is a popular commodity. It currently has the highest harvested acreage among all of the vegetables grown in Florida. In an attempt to expand sweet corn production in Florida, a sweet corn variety trial was conducted in northeast Florida at the University of Florida/IFAS Hastings Agriculture Extension Center (HAEC) in Spring 2020. Eight bi-color varieties including 'Superb MXR', 'Seminole Sweet XR', 'Obsession', 'Affection', 'Everglades', 'BSS-1075', 'BSS-8021', and 'CSABF13-698' were planted from seed on 2 March 2020 in Hastings. The seeds were planted on one acre in a randomized complete plot design with four replications on 40-inch rows with 6.125 inches interrow spacing. Harvest yields among the eight varieties ranged from 316–437 crates/acre (assuming 48 ears/crate). The difference between the lowest yielding variety ('BSS-8021') was significantly different from the highest yielding variety ('Affection'). The varieties with the highest percentage of ears that graded as U.S. Fancy after husked were 'Everglades' (90%), 'Affection' (86%) and 'Superb MXR' (83%). Continued efforts will be made to invigorate growers in northeast Florida to grow sweet corn and to establish appropriate planting strategies, variety selection, and market windows for this commodity.

Preserving agricultural land use in Florida is necessary for food and economic security, however, natural resources dedicated to farming are often targeted by developers. Vegetable cropping systems must continually evolve into sustainable, productive, and efficient land-use systems in order to maintain their place in rural Florida. New annual cropping schemes that incorporate high-value commodities are necessary for Florida to keep a competitive edge with other high productivity states. In the most recent Census of Agriculture, Florida ranked 5th in vegetable harvested acres after California, Idaho, Washington, and Wisconsin (USDA, 2017). Currently, sweet corn is the top producer among all vegetable commodities in Florida with 41,000 harvested acres in 2018 (USDA, 2019).

The Tri-County Agriculture Area (TCAA) in northeast Florida consists of St. John's, Putnam, and Flagler Counties. The primary cash crops are potatoes and cabbage. Sweet corn is a valuable commodity with a relatively short season that can boost economic viability as a secondary cash crop and increase land use efficiency if integrated strategically. Growers could integrate small acreage plots into their annual cropping scheme without losing any opportunities provided by their standard cash crop. Large commercial growers typically stagger their planting dates so with some strategic planning, they could incorporate sweet corn without any overlap of their primary cash crop. Cabbage growers, for example, could dedicate small acreage plots from their early cabbage to grow sweet corn in the spring, while potato growers could use plots dedicated to late potatoes for sweet corn plantings in the fall (see Fig. 1).

Research is currently underway to determine ideal planting dates for both spring and fall plantings of sweet corn in the TCAA. The goal of this project was to determine the most appropriate varieties to grow in the sandy soils commonly found in the TCAA.

Month	Potato Grower	Cabbage Grower
Jan	Potato	Cabbage
Feb	Potato	Cabbage
Mar	Potato	Sweet Corn
Apr	Potato	Sweet Corn
May	Potato	Sweet Corn
June	Cover Crop	Cover Crop
July	Cover Crop	Cover Crop
Aug	Cover Crop	Cover Crop
Sept	Sweet Corn	Cabbage
Oct	Sweet Corn	Cabbage
Nov	Sweet Corn	Cabbage
Dec	Potato	Cabbage

Fig. 1. Proposed Annual Crop Rotation Scheme for Incorporating Sweet Corn

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Specific objectives were to 1) identify days to maturity for each variety grown in a specific season and climate; 2) measure total harvest yields and grading quality for each variety; and 3) measure average weights of the ears, both husked and unhusked.

Materials and Methods

Sweet Corn Planting. Field experiments were conducted using eight different commercial bicolor sweet corn varieties grown during Spring 2020 at the Hastings Agriculture Extension Center. The seeds were provided by four different companies as shown in Table 1. They were planted in raised beds using a 4-row Monosem vacuum planter on 40-inch centers with an in-row spacing of 6.1 inches, resulting in a planting density of 25,602 plants per acre (A). Four replications of each variety were planted in a randomized complete block design. Each subplot consisted of four 100-ft rows. Sweet corn was planted in seepage irrigation fields on 2 Mar. 2020, with a total planted area of 0.84 A. The nutrient scheme consisted of a preplant application and three side dressings totaling 200 N–50 P–200 K (lb/A) . Specifics associated with soil preparation, irrigation and management of pest, weeds and diseases followed standard grower practices.

Sweet Corn Harvest and Evaluation. The harvest date was determined for each variety based on visual field observations such as kernel fill and plumpness as well as texture and taste. At harvest, the primary (top) ears in the central 30-ft of the middle two rows in each subplot were picked by hand, counted and weighed to get the total unhusked weight. A 30-ear subsample for each subplot was husked, weighed (husked weight) and graded as Fancy. Fancy ears were well-trimmed, well-developed, and free from decay, disease, and insect damage (USDA, 1992). We assigned all husked cobs that were well-covered, filled with plump, milky kernels and ≥ 6 in long as Fancy. Results were statistically analyzed using ANOVA followed by mean separation using Tukey's HSD test at $P < 0.05$.

Table 1. Participating seed companies and their affiliated sweet corn varieties.

Seed Company	Affiliated Varieties
Seminis	Affection, Obsession
Crookham	Everglades, CSABF13-798
Illinois Foundation Seeds Inc.	Seminole Sweet XR, Superb MXR
Syngenta	BSS-1075, BSS-8021

Results and Discussion

Weather conditions were optimum for crop development in this location. Daily air temperatures were predominantly within the optimum range for growth (60 to 80 ºF). Daily high and low air temperatures as well as rainfall events during the growing season were obtained from the Florida Automated Weather Network (FAWN) station located on-site and are shown in Fig. 2. Air temperatures dipped into the upper 30s shortly after planting, however, the high temperatures averaged 76 ºF during the first 7 d. Minimal rainfall was recorded during the first 30 days after planting (DAP). Weekly rainfall events accumulated 2.4 in of rain during the rest of the growing season. The earliest variety, 'Superb', was harvested 72 DAP and all remaining varieties were harvested 77 DAP.

Unhusked Evaluations. Total harvest yields for each variety in crates per acre are shown in Fig. 3 (assuming 48 ears/crate). The graphical depiction of results for unhusked evaluations incorporates all four replications for each variety except for 'Superb' and 'CSABF13-698', which are based on the average of three replications. The fourth subplots for these varieties were planted along a tree line and results were dismissed since most of the ears were decimated by raccoons. While 'Affection' had the highest average yield (437 crates/A) and the lowest variance among all four replications, there was no significant difference among the top producing 7 varieties. 'Affection' did have a significantly higher yield than 'BSS-8021'. However, 'Affection' had

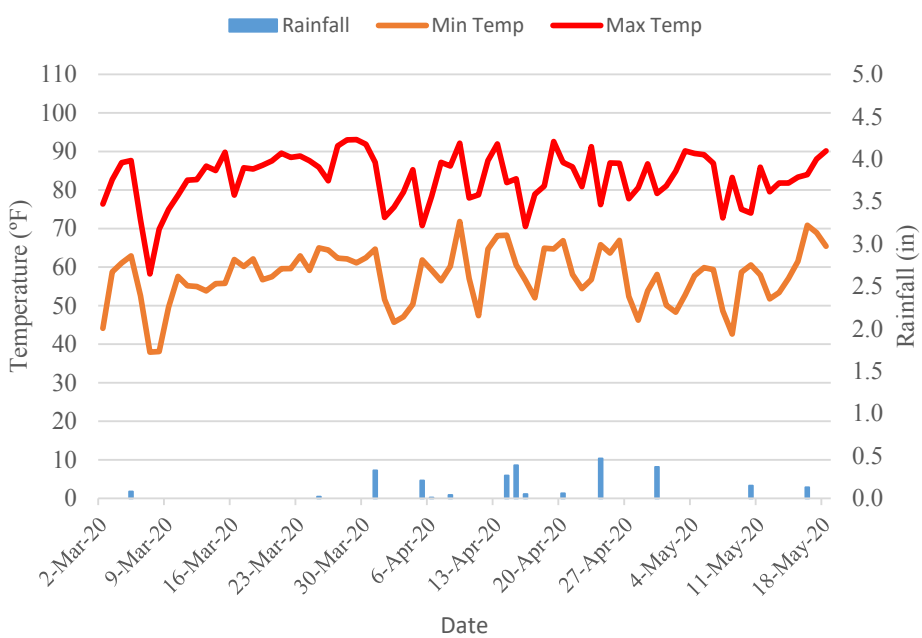

Fig. 2. Daily Minimum and maximum air temperature and rainfall events recorded by the Florida Automated Weather Network station in Hastings, FL.

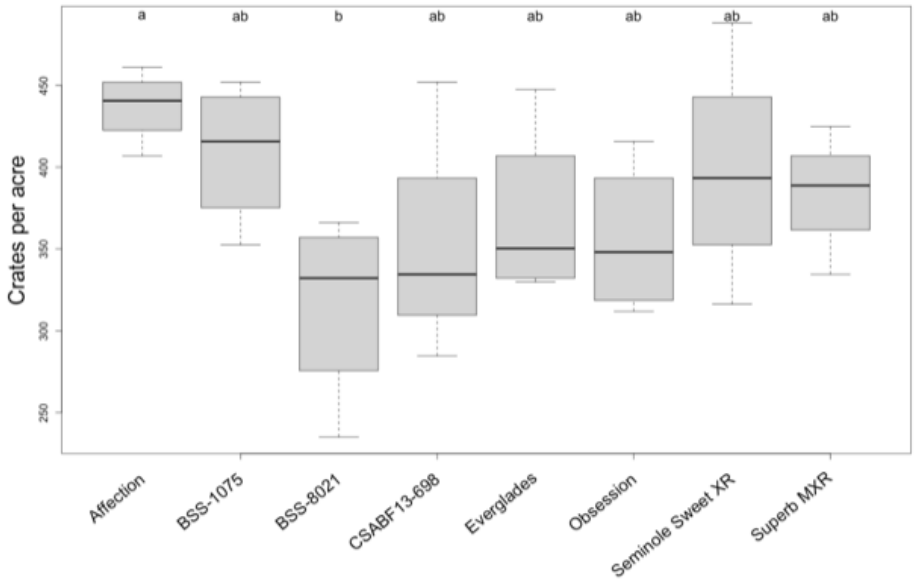

Fig. 3. Total marketable yields represented as crates per acre (letters on top represent significant differences according to the Tukey test at the 0.05 confidence level). Four replications are included for all varieties, except 'Superb' and 'CSABF13-698' where three replications are represented.

the lowest average unhusked per ear weight indicating that the ears and/or kernels were smaller than the other varieties (Fig. 4).

Husked evaluations. The average husked weight ranged from 0.19–0.65 lb/husked ear. 'BSS-1075' and 'Affection' had significantly lower average weight/husked ear (Fig. 5). The two hybrids from Crookham, 'Everglades' and 'CSABF13-698', had the highest per husked ear weight, although these were not significantly different from 'Obsession', 'Superb MXR', 'Seminole Sweet XR', or 'BSS-8021'. The percentage of the husked ears that graded Fancy for each variety are shown in Fig. 6. Although there were no significant differences among the varieties in regard to Fancy grades, 'Affection' and 'Everglades' had the highest average of Fancy grades and ear quality was consistently high among all four replications for these two varieties.

Conclusion

Although 'Affection' and 'BSS-1075' had the highest average harvest yields, there was no significant difference among the top seven varieties. Both 'Affection' and 'Everglades' were noted for their consistency in producing top grade or 'Fancy' ears and also for their excellent eating quality. Further research is planned that will expand the variety trials and provide data from multi-year cropping seasons in both the TCAA and selected sites in southern Georgia. Ideal planting dates within the TCAA need to be established for both spring and fall planting seasons.

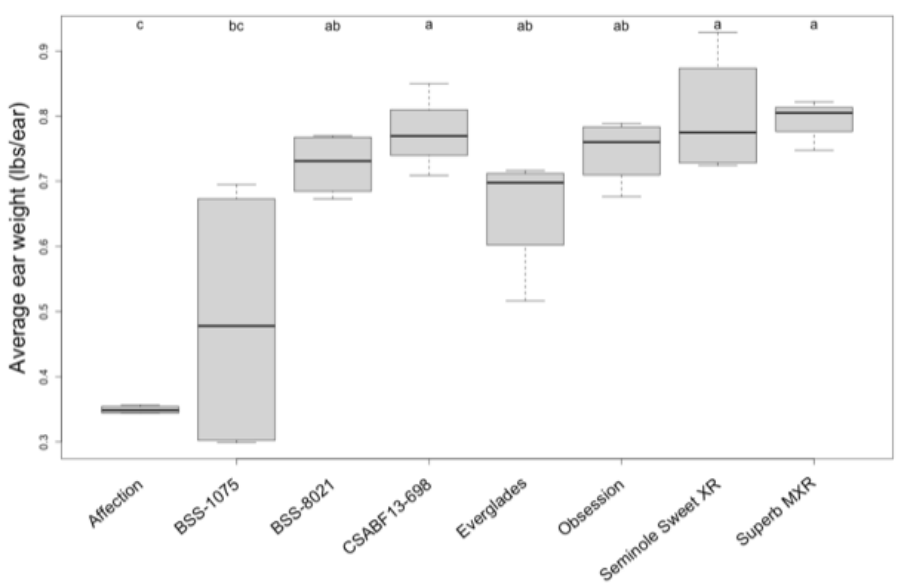

Fig. 4. Average unhusked ear weights (letters on top represent significant differences according to the Tukey test at the 0.05 confidence level).

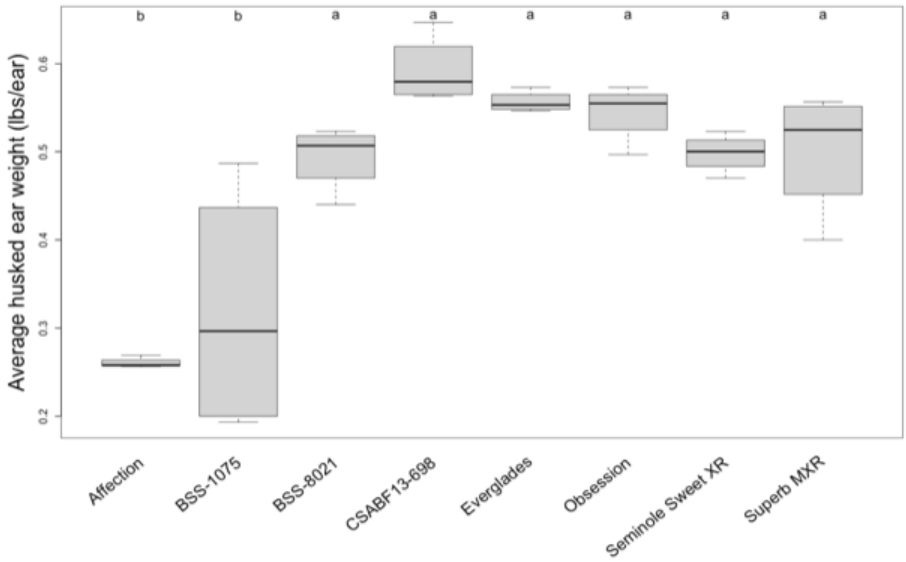

Fig. 5. Average husked ear weights (letters on top represent significant differences according to the Tukey test at the 0.05 confidence level).

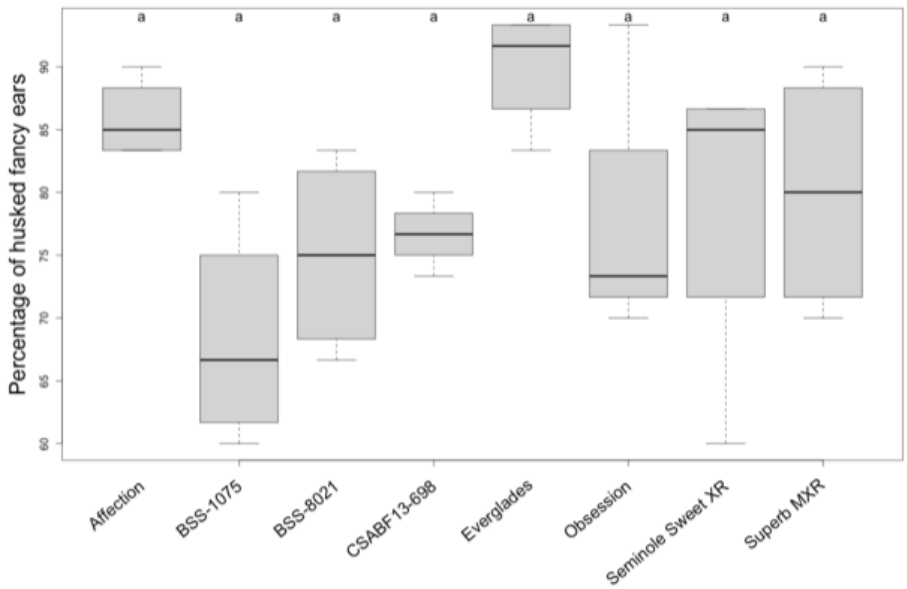

Fig. 6. Percent of husked ears rated as fancy grade according to USDA standards (letters on top represent significant differences according to the Tukey test at the 0.05 confidence level).

The ultimate goal of this research is to determine the most successful planting strategy for sweet corn in north Florida and to invigorate farmers in the TCAA to grow this commodity as a secondary cash crop behind cabbage in the spring or before potatoes in the fall.

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—Scientific Note—

Promotion of Hops as an Alternative Crop In Florida using Group and Mass Media Extension Approaches

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Additional index words. alternative crop, craft beer, craft brewery, *Humulus lupulus*, social media

Driven by the recent craft beer movement, hop (*Humulus lupulus* L.) production is currently expanding into nontraditional hop producing states. Our goal is to establish hops as a viable alternative crop in Florida. At the UF/IFAS Gulf Coast Research and Education Center, we built our first research hop yard in 2016. Over the last four years, our multidisciplinary team conducted a series of field experiments to select hop cultivars suitable forour climate, optimize crop management practices, develop pest management recommendations, and determine production costs. To disseminate these research results effectively, we first defined our target audiences. They were commercial growers, craft brewers, master gardeners, home gardeners, homebrewers, extension agents, and researchers. We used two approaches in our extension program: group and mass media approaches. We opted to add social media in the mass approach to communicate with more people of the target audience population.

In the group approach, we conducted field days, presentations at grower and scientific meetings, and sensory evaluations in the field. In the last four years, we organized nine field days, reaching a total of 601 participants. The field day event in May 2019 had 110 participants. Of 39 participants who completed our survey, 100% increased knowledge of hops, and 91% increased interest in growing hops. With support from local craft breweries, we also organized five tasting events of beer brewed with our hops. Seven types of beer were brewed with our hops and all were well-received by consumers.

In the mass media approach, we delivered our research findings via Facebook, YouTube, newsletters, magazine articles, extension publications, and conference papers. We published 189 posts on our Facebook page, which reached 38,342 people. Facebook expanded our audience's geographical distribution. Currently, there are 326 followers from 37 countries, of which 57%, 18%, 7%, 7%, and 6% are from the US, Brazil, Colombia, Australia, and Italy, respectively. On YouTube, we published 14 videos related to hops, which received more than 1700 views.

These extension activities facilitated the dissemination of research results to larger and diverse audiences. Field day participants gained hands-on experience, whereas Facebook was particularly useful to expand our target audience. In Florida, it is estimated that hop production increased from 0 to more than 30 acres. The use of both group and mass media approaches effectively helped promote hops as an alternative crop in Florida and other subtropical regions.

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—Scientific Note—

Lettuce in Advanced Production Systems as an Early Fall Alternative Crop

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Additional index words: crop production, lettuce

The objectives of this research are to determine if lettuce can be harvested in early fall using "advanced production practices" such as plasticulture, drip and transplants (Barrett et.al 2019) to attain attractive market prices <https://www.ams.usda.gov/market-news> often prevailing during the October/early November market window and if producing lettuce transplants for "advanced lettuce production" in a cooler region, specifically Blairsville, GA, increases the probability of less seedhead (seeder) emergence and ultimately higher yield.

In a 4 Nov. 2019 harvest (Fig. 1.) at the University of Florida/IFAS Hastings Agricultural Extension Center, three of four romaine lettuce cultivars resulted in acceptable yields in the October/early November market window. Two "Spanish greens" lettuce cultivars showed promise.

Work needs to be expanded to additional cultivars and higher plant population should be evaluated for "Spanish greens" cultivars. Cost of production and rotational crops on the "advanced lettuce production system" should be explored.

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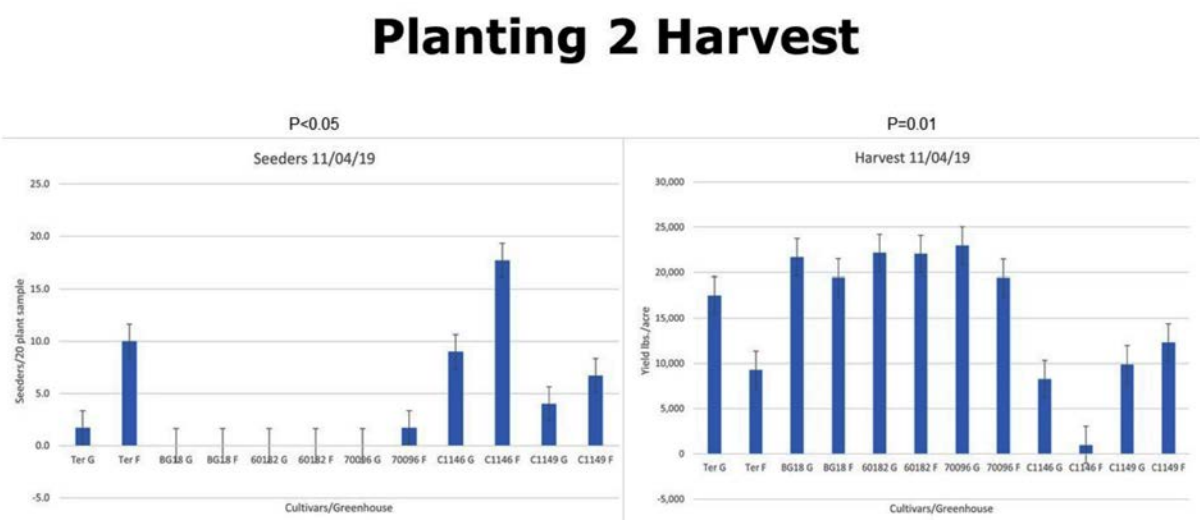

Fig. 1. Seeder formation and yield of six lettuce cultivars from Florida (F) and Georgia (G) grown transplants.

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—Scientific Note—

Field Evaluation of a New Mechanical Strawberry Gripper Developed for Autonomous Harvesting

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Additional index words. *Fragaria ×ananassa*, mechanical harvest, postharvest fruit quality

Strawberry (*Fragaria ×ananassa* Duch.) harvesting is a highly labor-intensive operation that represents the most expensive component of the strawberry production cost. Furthermore, labor shortages and increasingly labor costs are shrinking profit margins and creating uncertainty in the strawberry industry. Autonomous harvesting has the potential to solve these serious labor issues.

In this study, we designed a mechanical gripper with a scooping-pulling mechanism and constructed the gripper using 3D printing (Fig. 1A and B). The objective of this study was to evaluate the performance of the gripper and its impact on plant and fruit damage. When the gripper was placed in the right position, the strawberry picking success rate was nearly 100%. In a field evaluation experiment, we harvested fruits of three major strawberry cultivars ('Florida Radiance', 'Florida127', and 'Florida Brilliance') by handpicking or using the mechanical gripper throughout the growing season (Fig. 1C). To assess the impact of mechanical harvesting on plant stress, growth, yield, and fruit quality, we performed numerous growth and postharvest fruit quality measurements, including canopy size, canopy temperature (thermographic imaging), leaf chlorophyll index, normalized difference vegetation index, fruit Brix, fruit firmness, and fruit visual rating. Regardless of cultivars, mechanical picking did not affect canopy growth and plant stress levels. Marketable yield was 24.6 and 24.8 $t \cdot ha^{-1}$ for handpicking and mechanical picking, respectively, demonstrating no impact of mechanical picking on plant productivity. Although mechanical picking did not affect fruit firmness and Brix, it increased postharvest fruit visual damage by 15%.

These results suggest that the gripper designed in this study can successfully pick strawberries with minimal damage on plant growth and productivity. Some modifications are needed in the picking mechanism or material of the gripper to minimize mechanical fruit damage.

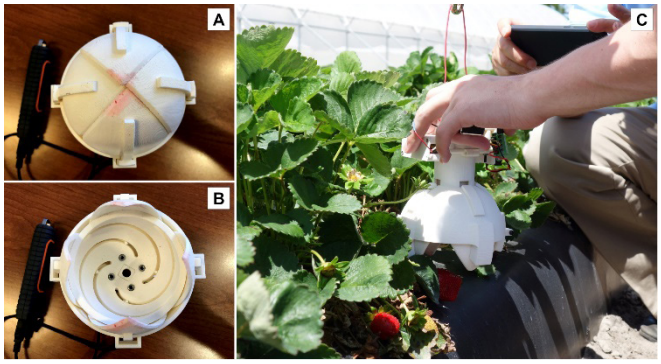

Fig. 1. A mechanical strawberry gripper with a scooping-pulling mechanism: closed position (A), open position (B), and harvesting action (C).

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—Scientific Note—

Characterization of Leaf Gas Exchange in Hops Grown In the Subtropical Production System with Supplemental Lighting

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Additional index words. abiotic stress, acclimation, alternative crop, *Humulus lupulus*, photosynthesis, stress adaptation

Subtropical climatic conditions with supplemental lighting allows two growing seasons of hops (*Humulus lupulus* L.) a year. In central Florida, the spring season is from mid-February to early June, followed immediately by the fall season, which ends by early December. Plants also undergo a hot and humid rainy season from June to September.

To evaluate the physiological adaptation of hops to this unique production system, we characterized leaf gas exchange of 'Cascade' hops grown in central Florida over two growing seasons (Spring and Fall 2019). Plants were established in the field with a 6-m high trellis in February 2018. Leaf gas exchange was measured during five developmental stages: bine emergence, bine elongation, lateral branching, cone-development, and cone maturation. Leaf gas exchange showed different patterns between the two seasons and varied considerably across developmental stages. In the spring season, net CO_2 assimilation rate (A) was highest during the bine elongation stage (21.7 μmol·m^{-2}·s^{-1}) and gradually declined thereafter until the cone maturation stage (13.1 μmol m^{-2} s^{-1}). In the fall season, A was highest during the bine emergence stage (21.8 μmol·m^{-2} s^{-1}), despite high heat and humidity levels in early August. A rapidly declined thereafter, recovered to the initial level at the cone development stage, then declined again at the cone maturation stage (14.6 μmol·m^{-2}·s^{-1}). Stomatal conductance showed similar trends as A, indicating stomatal limitation to photosynthesis along the developmental stages in both seasons. When averaging across developmental stages for each season, A was similar in both spring (17.7 μmol·m^{-2}·s^{-1}) and fall seasons (16.7 μmol·m^{-2}·s^{-1}). By contrast, stomatal conductance increased by 20% in the fall season compared to the spring season (0.319 vs. 0.390 mol·m^{-2}·s^{-1}) HO_2, indicating enhanced stomatal opening by higher temperatures in the fall season (28.5 to 33.6 °C vs. 29.5 to 35.5 °C). In the spring season, water use efficiency (WUE) was relatively low during the rapid biomass accumulation period, including bine elongation, lateral branching, and cone development stages, compared to bine emergence and cone maturation stages. This decrease in WUE was due to an increase in transpiration rather than to a reduction in A.

These results suggest that hot and humid subtropical climatic conditions are not a limiting factor for gas exchange of hop plants. The fact that the most active gas exchange occurred during bine regrowth in early summer is particularly notable. It is also important to note that hop plants can maintain the same level of gas exchange in both spring and fall seasons despite different environmental conditions. Changes in gas exchange activities across developmental stages within each growing season are likely associated with development-specific growing behaviors. Overall, hop plants show high physiological adaptation to subtropical climatic conditions.

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—Scientific Note—

Globe Artichoke Variety Trials Using Controlled-release Fertilizer In Northeast Florida

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Additional index words. artichoke, controlled-releases fertilizer

Introduction and Background

Globe artichokes (*Cynara scolymus*) are not a commonly grown crop in the state of Florida. California produces 99% of the market supply as its growing conditions are like the Mediterranean environment where this plant is indigenous. Artichokes have a moderate salt tolerance, which is important for production in coastal areas such as St. Johns County, FL. The globe artichoke is a thistle plant in the Asteraceae family. The time of maturity from seed planting to harvest can take four to six months. In the 2019 season (March–October), a carton of California-grown artichokes ranged from $15 to $63.50, and up to $58.50 in 2020, with fresh market cartons typically being 24 s and 36 s with regard to head count. With little to no competition on the East coast, there is an opportunity for Florida farmers to diversify with this alternative row crop.

In Florida, the crop is treated as an annual, and typically planted in the early fall as seedlings. For bud development to occur, the plants need to experience adequate chilling (depending on the variety) with temperatures below 50 degrees Fahrenheit. For this reason, growth regulators, such as Gibberellic acid (GA) are used to assist the plant in producing the same hormones as if they had experienced adequate chill. At maturity, the plant can easily reach 4 feet tall and wide.

Materials and Methods

The variety trial took place in Hastings, St. Johns County, FL. at the University of Florida/IFAS Hastings Agriculture and Education Center. The half-acre trial was planted on Alfisol soils using a randomized split-block design representing two planting dates (phase 1 vs. phase 2): 7 Oct.2019 and 11 Nov. 2019. Each block contained six varieties replicated four times, with 12 plants per replication. The varieties/cultivars in the trial include 'Colorado Red Star', 'Green Globe Improved', 'Green Queen', 'Imperial Star', 'Opal', and 'Purple Romagna'. Transplants were installed on 72" black plastic (48" row width) with double-line drip irrigation. Artichoke transplants were planted 36" apart with beds raised 6". A 16–6–11 controlled-release fertilizer blend with minors from Harrell's was used pre-plant at 1250 lbs/acre. The plant growth regulator ProGibb (Gibberellic acid) was applied at 20 ppm the fourth true-leaf stage, with a second application 10 d later.

Results and Conclusions

'Green Globe Improved', 'Green Queen', and 'Imperial Star' had the most impressive yields of the six varieties trialed. While 'Colorado Red Star' and 'Opal' were deemed the favorites by color and appearance in a farmer's market survey, they did not have adequate yield. 'Purple Romagna' failed to produce buds on most of the plants and does not appear to be commercially viable for the area based on this season. When comparing the two phases of varying planting dates, there did not seem to be a significant difference in marketable yield. However, the yield was higher in the November planting date, despite 15% yield loss due to spider mites, blossom end rot and *Pectobacterium*. The October planting phase was damaged by spider mites and *Pythium* due to the warm soil and air temperature at planting, in addition to black plastic mulch. Figure 1 shows marketable yield data per cultivar and planting date.

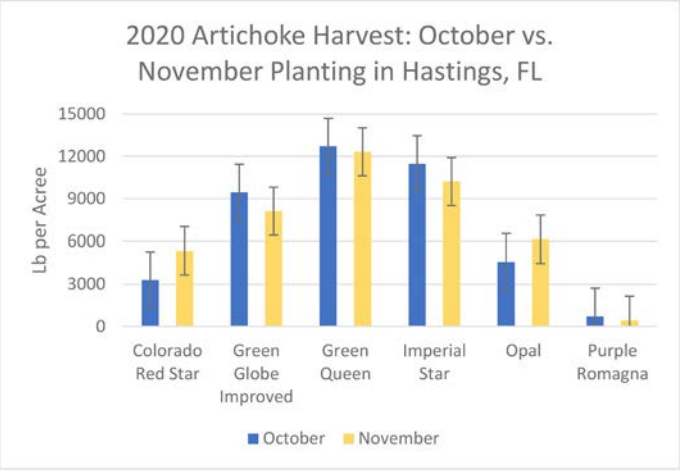

Fig. 1. Marketable yield of 6 artichoke cultivars planted in October vs.November.

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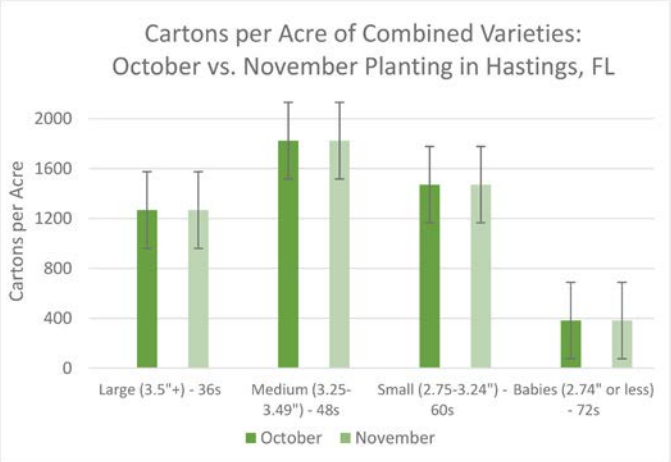

Fig. 2. Yield across all cultivars for two planting dates, expressed as 4 size categories.

Most of the buds were in the "Medium" size range, followed by "Small." With 800 cartons per acre being considered good or adequate, there were still enough "Large" buds to be comparable to typical California production yields (Fig. 2).

Suggestions for Future Studies

There is limited research available for nutrient management of artichoke production, especially within the United States. As farmers gain interest in growing this crop, more nutrient rate work is needed, as we are currently following California practices. We also feel that the Gibberellic acid might have been applied prematurely, which could explain the number of small buds.

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—Scientific Note—

Developing Interspecific Bridges between *Cucurbita pepo* and *C. moschata*

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Additional index words. cucurbit, embryo rescue

Interspecific hybridization was performed among 11 *C. moschata* and 4 *C. pepo* accessions. One hybrid (*C. moschata* × *C. pepo*) of the 24 crosses showed compatibility and immature seeds developed to adult plants via embryo rescue. The progeny of the derived crosses of *C. moschata* × (*C. moschata* × *C. pepo*) and (*C. moschata* × *C. moschata*) × (*C. moschata* × *C. pepo*) were introgressed with *C. pepo* and *C. moschata* to produce a set of bridge materials with different amounts of *C. pepo* genetic background e.g. 25%, 31.25%, 62.5%, 81.25%, and 90.625%, respectively, some of which held elite traits and potentials of disease resistance.

Interspecific hybridization is an important traditional breeding method for introgressing novel genes and widening genetic variations. *C. pepo* is the most diverse species for fruit characteristics among the five domesticated *Cucurbita* species and holds elite yield traits, while *C. moschata* is more tolerant to heat and resistant to pests. Hybridization between the two species is possible, but difficult due to incompatibility issues (Rakha et al., 2012). To develop bridges between these two species, we have tested 24 interspecific crosses for compatibility. By combining practical embryo rescue techniques with introgression across generations, the bridges were created and selected for elite characteristics.

Eleven *C. moschata* and 4 *C. pepo* cultigens were used to produce 24 interspecific crosses in 2015. For continuous introgression and breeding purposes, 5 of the above parental *C. moschata*, some of which are of crown rot and/or powdery mildew resistance genes and other 4 *C. pepo* cultigens including the very popular 'Yellow Crookneck', 'Early Prolific', and 'Table Queen Acorn' were involved. To overcome the interspecific barriers, immature embryos were rescued by culturing on E20 or E21 medium (Nuñez-Palenius et al., 2011).

Findings

Of all the 24 initial crosses, one [C (*C. moschata*) × J (*C. pepo*)] was successful. Using the C × J (F_1) as the male parent, a three-way cross of O (*C. moschata* with crown rot resistance) × (C × J) (F_1) and a double cross of [Q (*C. moschata* with crown rot resistance) × D (*C. moschata)*] (F_1) × (C × J)(F_1) were developed, resulting in the progeny containing 25% *C. pepo* genetic background. These progeny, when introgressed with *C. pepo* or *C. moschata*, were generally more compatible with higher fertility and viability compared to the initial cross of C × J. Some of the materials grew to normal plants via embryo rescue. A set of bridge materials with 25% to 90.625% *C. pepo* background were created, and some of them showed elite yield traits e.g. bush type, short internodes, an increase in female flowers, unique fruit color, and potential resistance to diseases. The introgression strategy employed in this study could be adopted to produce interspecific hybrids using other *Cucurbita* species. With the introgression of the crown rot resistance, we would be one step closer to introduce resistance genes into the extremely susceptible commercial summer squash (*C. pepo*).

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—Scientific Note—

The Genetic Diversity of the Genus *Lactuca* for Resistance to Fusarium wilt

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Additional index words. breeding, disease resistance, wildtypes, lettuce

Fusarium wilt of lettuce (FOL) (*Lactuca sativa* L.) is a soil-borne disease caused by the fungus *F. oxysporum* f.sp. *lactucae*. The fungus enters plants via natural apertures or wounds on roots and causes a reddish-brown necrosis in the taproot's vascular tissue. Subsequent decay within root vascular tissue inhibits water and nutrient transport, resulting in wilting, stunting and chlorosis of leaf tissue (Scott et al., 2012). FOL may cause widespread crop losses, particularly in favorably high soil temperatures, and can persist for years in infected fields once the fungus produces resilient resting structures called chlamydospores. In the US, the pathogen was reported first in California in 1990, Arizona in 2001, and Florida in 2017. It may now pose a threat to the $80 million per year lettuce industry if FOL increases in the Everglades Agricultural Area (EAA). The lack of control methods against this disease makes breeding for resistance a top priority. Several cultivated lettuces in the USDA-Germplasm Resources Information Network (GRIN) have resistance against FOL, however, many are only partially resistant and to our knowledge, no resistant wildtype or primitive *Lactuca* have been identified. Additional resistance is needed to increase the genetic pool available for breeding. The purpose of this study was to identify potentially novel sources of resistance within cultivated and wildtype *Lactuca* accessions from the center of origin.

Two augmented randomized complete-block design experiments were conducted in a dedicated greenhouse at the Everglades Research and Education Center between January and March of 2020. Five plants per plot of four-week-old seedlings for each accession were inoculated with isolate FOL #51 using the method described by Murray et al. (2020). This isolate was previously identified as the EAA's most virulent race 1 isolate. Root discoloration incidence (RDI) and severity (RDS) were recorded after 30 d post-infection using a 0–5 scale, where 0 = no taproot discoloration to 5 = plant death. Root tissue samples of non-symptomatic plants were plated on Komada's Semi-Selective Media and identified by conidia morphology to confirm the pathogen's presence inside asymptomatic taproots. The first experiment consisted of 48 accessions obtained from USDA-GRIN, including 35 *L. sativa* and 13 *L. serriola*, as well as four controls: 60182, a University of Florida breeding line resistant to FOL race 1; 'Lolla Rossa' [Plant Introduction (PI) 617943], and 'Dark Lolla Rosa' (PI 667690) with partial resistance; and the susceptible 'Chosen'. The second experiment consisted of 111 accessions from the University of California, Davis germplasm collection that originated from several countries in *Lactuca*'s Mediterranean center of origin, including, 98 *L. serriola*, 5 *L. saligna*, 8 *Lactuca spp*. accessions, and the aforementioned controls. Analysis of variance was conducted to test the effect of accessions on RDI and RDS using SAS software, Version 9.4 (SAS, Cary, NC). Post-hoc analysis was conducted with Dunnett's two-tailed t-test to determine significant differences between each accession and each of the four controls.

Significant differences were found among tested accessions in experiments 1 and 2 for RDI and for RDS ($P < 0.0001$). In the first experiment, 20 accessions were without root discoloration or foliar symptoms. However, when these taproots were plated onto Komada's Media, only one, PI 165063, did not develop any FOL colonies and is considered completely resistant. In the second experiment, 27 accessions of wildtypes were without foliar symptoms or root discoloration, and of those, only five *L. serriola* accessions did not grow FOL colonies when plated on Komada's media.

Several *L. sativa* accessions have been described with resistance to race 1 for isolates in California and Arizona, including the romaine cultivars 'Costa Rica No.4', 'Caesar', 'King Henry', 'Slugger', 'King Louie', and 'Valmaine'; as well as leaf cultivars 'Lolla Rossa' and 'Red Rossa' (Matheron and Porchas, 2010; Scott et al., 2010; Scott et al., 2012). However, FOL was recently discovered in Florida and these experiments utilized a

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novel isolate obtained from local fields. The leaf cultivar 'Dark Lolla Rossa' and romaine breeding line 60182 showed little to no disease in these experiments and can be considered resistant to FOL. No relationship between origin and resistance to FOL can be established for *L. sativa,* because only one resistant PI from Turkey has been discovered. However, the five wild-type (*L. serriola*) resistant accessions were all collected from the Caucasus region of Azerbaijan. Although there is genetic diversity for race 1 of FOL in the genus *Lactuca*, screening for wildtypes is still needed to have alternative sources of resistance that could be pyramided, especially if resistance is controlled by different loci.

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—Scientific Note—

Resistance to Cucurbit Leaf Crumple Virus In Squash

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Additional index words. breeding, disease resistance, whitefly transmitted viruses

The cucurbit industry in Florida is under threat from damaging outbreaks of Cucurbit leaf crumple virus (CuLCrV), a whitefly transmitted virus. Epidemics caused by CuLCrV have intensified over the last few years causing some growers to abandon their fields or face greatly reduced yields. Management of CuLCrV is difficult due to the development of pesticide resistance in whitefly populations, lack of resistant commercial cultivars, as well as limited knowledge on the biology of the virus and its interaction with plant hosts. Cultural management options reply on suppression of whitefly populations using reflective mulches, removal of volunteer plants and crop rotation; however these are not wholly effective, especially under high whitefly pressure.

The goal of the current study was to identify squash germplasm resistant to CuLCrV under field conditions. One hundred and seventy accessions of squash (*Cucurbita* spp) were grown at the North Florida Reseach and Education Center–Suwanee Valley Experiment Station, Live Oak, FL. Twelve seedlings per accession were transplanted on plastic mulch in Sept. 2019, and data on disease severity were collected every week for 5 weeks. Molecular diagnosis to confirm infection with CuLCrV was done using PCR or RPA. Area under disease progress curve (AUDPC) was calculated to determine the rate of disease progression. It ranged from 0–1852 across all accessions. Forty-three accessions showed moderate disease severity (AUDPC = 100–400), while the majority (n = 116) of the accessions were highly susceptible, including the commercial cultivars (mean = 1286). However, 11 accessions (8 *C. pepo* and 3 *C. moschata*) did not develop any symptoms (asymptomatic). Identification of these accessions presents the fist step toward development of commercial squash cultivars resistant to CuLCrV for growers.

Further studies are underway in the greenhouse to confirm resistance.

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—Scientific Note—

QTL-seq for Rapid Mapping of Genomic Regions Associated With Zucchini Yellow Mosaic Virus Resistance In Squash

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Additional index words. cucurbit, genetic markers, pest resistance

Development of Zucchini Yellow Mosaic virus (ZYMV) resistant squash and pumpkin cultivars using traditional breeding approaches relies on rigorous and resource-intensive phenotypic assays. QTL-seq, a whole genome resequencing based bulked segregants (BSA) is a powerful tool for mapping quantitative trait loci (QTL) associated with a trait. In the current study, 4 QTLs significantly associated with ZYMV resistance ($P < 0.05$) were detected in chromosomes 2, 4, 8 and 20 using QTL-seq method. 12 Indel markers were developed within the QTL intervals using BatchPrimer3. Out of the 12 markers, one marker linked to the QTL in chromosome 20 was found to be polymorphic between the resistant and susceptible parents used in the study. The study demonstrated the efficiency of QTL-seq in identifying QTL's associated with ZYMV resistance. Current efforts involve validating the above identified polymorphic marker in diverse genotype and also developing additional markers that will compliment phenotypic selection in future breeding programs.

ZYMV is one of the major yields limiting factor in *Cucurbita* production worldwide. Natural source of resistance to ZYMV has been identified in an unadapted *Cucurbita moschata*, 'Nigerian Local'. Identification of QTL associated with resistance and molecular tagging of resistant loci would assist in marker assisted pyramiding of resistant alleles in commercial cultivars through marker assisted selection.

A mapping population was developed by crossing 'Nigerian Local' (ZYMV resistant) X 'Butterbush' (susceptible). Briefly, controlled crosses were made to produce the F_1 generation. These were selfed to produce the F_2 or the mapping population. The F_2 population was mechanically inoculated with ZYMV and screened phenotypically to select the 10 most resistant and the 10 most susceptible individuals. DNA from the 10 most resistant and the 10 most susceptible individuals were pooled together for preparation of resistant and susceptible bulk, respectively. Resistant and susceptible parents, and resistant and susceptible bulks were sent for whole genome sequencing and sequencing results were used for variant calling and QTL analysis.

Grouping of F_2 populations as resistant and susceptible served the purpose of phenotypic screening. QTL seq analysis using QTL-seqr identified 4 QTL's associated with ZYMV resistance on chromosomes 2, 4, 8, and 20. Indel markers were developed from the vicinity of QTL regions. Markers were checked for polymorphism and one marker in chromosome 20 could confidently discriminate resistant and susceptible individuals. Future work involves identifying Single nucleotide polymorphism markers to develop higher confidence in marker assisted selection for ZYMV resistance development.

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—Scientific Note—

Alternative Fumigant Application Strategies to Manage Plant Parasitic Nematodes In Vegetable Crops

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Additional index words. disease and management

Pre-plant soil fumigation has been a fundamental treatment in intensive Florida plasticulture production since the 1960s. Since the phase-out of methyl bromide (MBr) in 2005, the incidence and severity of nematode-infested fields have increased in vegetable producing systems and a soilborne pest and disease management program with reliable and consistent efficacy remains elusive. Unlike MBr, alternative fumigants have lower vapor pressure and higher boiling point, and consequently, poorer vertical and horizontal distribution in soils. A point of concern when making a pre-plant fumigation is the depth at which the fumigant is applied. Previous research in cotton and strawberry has shown that root-knot nematodes [(RKN) *Meloidogyne* spp.] and the reniform nematodes (*Rotylenchulus reniformis*) can inhabit very deep soil profiles, below the depths to which any of the current shank- or drip-applied fumigants diffuse. Because these nematodes at greater depth can rapidly recolonize fumigated soils, a reassessment of fumigant application depth for plasticulture systems is needed. The objective of this study was to evaluate the efficacy of deep, shallow, and shallow plus deep drip (combo) fumigation for RKN and reniform nematode management.

A field experiment was performed at the North Florida Research and Education Center in Quincy during fall 2019. The study evaluated seven treatments: a non-treated control and two fumigants [1,3-dichloropropene (1,3-d) and dimethyl disulfide (DMDS) at 169 L/ha and 375 L/ha, respectively) applied at three different depths {shallow drip (2.5 cm), deep drip (38 cm), and a combo [shallow and deep, (2.5 and 38 cm)]} through a single drip tape buried at each depth. All experimental plots were covered with white-on-black totally impermeable film mulch prior to fumigation. Fifteen cantaloupe (cv. Athena) seedlings were transplanted in each plot 3 weeks after fumigation. The experiment was established in an area known to have naturally occurring RKN and reniform nematode populations. The experimental design was a randomized complete-block design with 4 replications.

Marketable fruit yield was estimated for each plot at fruit maturity. Three 6-inch deep soil core samples and five root systems were collected from each plot for nematode quantification and root galling index (RGI) rating. Analysis of variance (ANOVA) was performed using SAS® software, version 9.4 (SAS, Cary, NC) to test the effect of fumigant, depth, and fumigant × depth interaction. The LD_CI macro was used to calculate the relative marginal effects (RMEs) of RGI rating and their 95% confidence intervals. Orthogonal contrasts were calculated to compare each of the fumigant treatments against the control. ANOVA indicated no significant differences ($P > 0.05$) for marketable fruit yield among treatments. However, a trend of greater yields was observed in the fumigant treatments compared the control, except for the 1,3-d applied at a deep depth. In general, DMDS shallow and 1,3-d deep resulted in a lower number of reniform nematodes in the soil, though, differences were not statistically significant ($P > 0.05$), and no differences in RKN count for both soil and roots were observed among treatments. Linear contrasts of RMEs for RGI showed that DMDS shallow and 1,3-d deep were significantly different ($P < 0.05$) compared to the control. Overall nematode populations were low in the trial area which resulted in variable data. These results may suggest that deep drip fumigation will not necessarily improve overall nematode control or crop yield.

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—Scientific Note—

Effect of Multispecies Cover Crop Mixture on Root Colonization by Arbuscular Mycorrhizal Fungi

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Additional index words. AMF inocula, multispecies cover crop mixture phosphorus, potato

The use of commercial arbuscular mycorrhizal fungi (AMF) inocula is a growing industry in agriculture, particularly in horticulture and landscaping (Chen et al., 2018). However, the benefits of amending soil with mycorrhizae, such as increased plant phosphorus (P) uptake and resistance to pathogens and reduced aluminum (Al) phytotoxicity, are highly variable due to soil conditions and crop management (Knerr et al., 2018). The number of spores in these commercial inocula can be more or fewer than what a company label specifies and spore viability is not always guaranteed (Evans and Snow, 2000). Also, AMF species in commercial inocula can compete with the pre-established natural AMF communities in the soil (Janousková et al., 2013). Depending on the outcome of the competition, commercial inocula can be ineffective for anastomosis which is the restoration of damaged fungal hyphae through hyphae fusion of the same or different AMF species (Zimmerman et al., 2009). Damaged hyphae may result from the action of AMF-suppressive bacteria in soil (Svenningsen et al., 2018). The purpose of this study is to reduce the reliance on inorganic phosphorus fertilizer in high-legacy-P potato cropping systems by increasing the population of naturally occurring AMF by planting multispecies cover crop (MSCC) mixture. Different AMF species colonize different crops so MSCC were used to screen pre-established AMF species in our research site. Our objectives were to evaluate the number of AMF spores and root colonization of MSCC and evaluate P uptake by MSCC.

A study was conducted at Hastings Agricultural Extension Center. The experimental design was a split-block design with 4 replicates of four treatments: MSCC; MSCC+AMF; MSCC+Fumigant; and a control. Two P rates of 0 and 100 lb/acre (112 kg/ha) per treatment were the subplots. The data presented compare only the control and MSCC treatments from summer 2019. The MSCC is composed of Sunn hemp [*Crotalaria juncea* (20 lb/acre)], Sorghum sudangrass a.k.a. Sudex [*Sorghum bicolor* × *S. bicolor* var. *Sudanese* (20 lb/acre)], and velvetbean [*Mucuna pruriens* (30 lb/acre)]. Velvetbean has a high invasion risk in southern and central Florida but not in the north <https://assessment.ifas.ufl.edu/>.

Our results indicated that MSCC had higher AMF root colonization (68.5%) compared to the control (31.7%). However, the number of AMF spores in the soil was not significantly different between treatments (Fig. 1). We expected to find a higher number of spores as root colonization increased. MSCC can take up to 0.4% of inorganic phosphorus, an average of 2.8 to 21.2 lb/acre

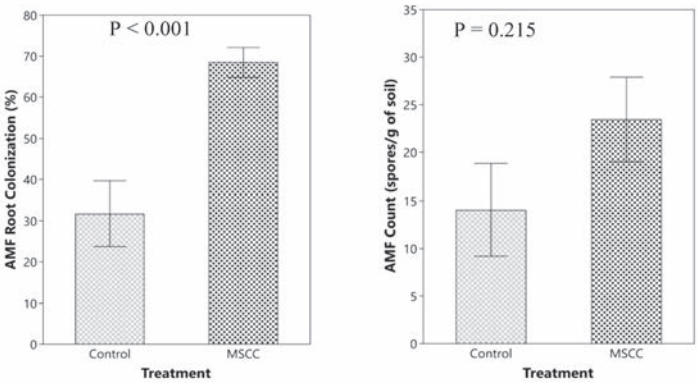

Fig. 1. The percentage of roots colonized by AMF (left) and the number of AMF spores (right) in the soil between in control and MSCC treatments. $P \leq 0.05$ indicates a significant difference between treatments.

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Table 1. Growth and macronutrient content of individual cover crop species within the multispecies cover crops (MSCC).

	Growth parameters				Macronutrient content					
	Height	Plant density	Shoot dry wt		N	P	K	Mg	Ca	S
MSCC	(ft)	(acre *1000)	(lb/acre)	(lb/acre)	%					
Sunn hemp	4.9	181	5159.7	5715	3.07	0.41	1.19	0.55	1.60	0.31
Velvetbean	3.7	23	646.8	625	2.07	0.43	1.44	0.52	1.21	0.27
Sudex	4.5	138	1290.8	1249	2.61	0.41	1.28	0.55	1.39	0.27

of P depending on the average shoot dry weight of the combined cover crops (Table 1). AMF inocula are naturally occurring AMF species vary depending numerous factors such as soil type, soil nutrients, previous crops, etc. and that commercial inocula often don't contain any of the naturally occurring species. Since naturally occurring species are adapted to the crops planted at their location, they may be much more effective than AMF species in commercial mixtures and commercial AMF mixtures may be totally ineffective depending on the AMF species included. Therefore, it is important to produce naturally occurring AMF inocula. Thus, future research will evaluate the AMF root colonization of 'Atlantic' potato following MSCC and assess its effect on tuber yield and yield components.

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—Scientific Note—

Post-planting Root Growth Dynamics and Morphology of Bare-root and Plug Strawberry Transplants and Their Impacts on Field Performance

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Additional index words. *Fragaria ×ananassa,* image analysis, ImageJ, rhizotron, root morphology, stand establishment

The use of plug transplants for strawberry (*Fragaria ×ananassa* Duch.) production has increased recently because their establishment in the field is quicker and does not require overhead irrigation compared with bare-root transplants. The most notable difference between the two transplant types is in their root systems. Plug transplants have alive and functional roots, whereas most fine roots of bare-root transplants are desiccated desiccated at the time of transplanting, resulting in a limited water nutrient uptake capacity.

We conducted a greenhouse experiment using a scanner-based rhizotron system to characterize initial root growth dynamics and morphology of bare-root and plug strawberry ('Florida Radiance') transplants. Each rhizotron had 2655 cm^3 of soil with a 23 × 30 cm scannable window on each side. The soil was collected from a research strawberry field and packed in the rhizotrons using the field bulk density. Rhizotrons are inclined at 30° to promote root growth on the bottom side window. Plants were grown over 42 days in the rhizotrons. We measured canopy and root projected area weekly via image analysis of overhead canopy and root scan images.

In bare-root transplants, most initial roots were desiccated before transplanting and decayed during the experiment. They developed new roots from the basal part of the crown tissue or tips of some initial roots, most of which were detected in the upper middle 15 cm of the soil profile. By contrast, plug transplants had relatively undamaged initial roots, which continued to grow and developed uniformly into the entire soil profile. Compared with bare-root transplants, plug transplants showed more rapid canopy growth, with canopy projected area increasing by 61% to 135% during the experiment period. Root growth showed different vertical root distribution between the two transplant types.

At the end of the experiment, although root projected area in the upper 15 cm was similar in both transplant types, plug transplants had 246% larger root projected area in the lower 15 cm than bare-root transplants. In a follow-up field experiment, plant growth and yield performance of bare-root and plug strawberry transplants were evaluated. The faster initial canopy development of plug transplants compared to bare-root transplants was observed, with up to 39% larger canopy projected area at 28 days after transplanting. Consequently, plug transplants produced 52% and 23% higher early and total season yields than bare-root transplants, respectively.

These results suggest that rapid establishment and improved fruit earliness of plug transplants is due partly to their functional initial roots that can continue to develop and quickly explore the soil for water and nutrients after transplanting.

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—Scientific Note—

QTL Mapping of *Phytophthora* Crown Rot Resistance in Squash

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Additional index words. *Phytophthora capsici*, squash breeding

Squash (*Cucurbita pepo* L.) is an important vegetable crop in Florida valued at over 30 million US dollars (USDA-NASS, 2020). Losses due to *Phytophthora capsici* Leonian are a major challenge throughout the state. *P. capsici* is the causal agent of *Phytophthora* crown rot in squash, is a disease exacerbated by frequent flooding in Florida. Cultural management techniques are ineffective under heavy disease pressure and the pathogen rapidly develops insensitivity to fungicides. Although host resistance is preferred, no commercial summer squash cultivars resistant to the pathogen are currently available. A breeding line, UF-PhytoR1, with high resistance to *Phytophthora* crown rot was developed at the University of Florida (Michael et al., 2019). This study was conducted to map QTL loci associated with *Phytophthora* crown rot resistance in UF-PhytoR1 and identify any linked molecular markers for application in marker assisted backcrossing.

Materials and Methods

An F_2 mapping population was developed from a cross between UF-PhytoR1 and 'Table Queen Acorn', an elite susceptible cultivar and subsequently selfed to produce $F_{2:3}$ seeds. For phenotyping, 12 seeds of each $F_{2:3}$ family and 10 seeds of each parent were sown in 4-inch pots and inoculated at 2–3 leaf stage with a virulent P. capsici strain by spraying 1.5 mL (vol.) of zoospore suspension (2.0×10^4 zoospore/mL) at the crown. Disease severity (DS) was recorded every 48 h starting 6 d post-inoculation (dpi) using a scale of 0 to 5 in which, 0 = no symptoms and 5 = plant death. Area under disease progress curve (AUDPC) values calculated from mean DS scores of each $F_{2:3}$ family were used in QTL mapping. Concurrently, genomic DNA extracted from each F_2 plant and the parents was genotyped with 522 squash single-nucleotide polymorphism (SNP) markers on a SeqSNP platform. A genetic map was constructed with polymorphic SNP markers between UF-PhytoR1 and 'Table Queen Acorn' by assembling them into linkage groups using Kosambi mapping function (LOD = 4.97, recombination fraction = 0.5) in R/onemap (Margarido et al., 2007). Finally, significant QTLs were identified by composite interval mapping using Harley-Knott regression with LOD thresholds of 4.14 determined with 10,000 permutations in *R/qtl2* (Broman et al., 2019).

Results and Discussion

On the last day of screening, all 'Table Queen Acorn' plants had died (mean DS score = 5) while all UF-PhytoR1 plants had a mean DS of 0.55 and exhibited no typical *Phytophthora* crown rot symptoms. Meanwhile, AUDPC scores of $F_{2:3}$ families ranged from 15.45 to 45.01 (mean = 35.39) and displayed a moderately left skewed normal distribution (Pearson coefficient of skewness = –1.105). A significant QTL region (*qtlpc*) spanning approximately 0.66MB in chromosome 13 was detected. Several candidate defense-related genes were found within the confidence interval of the QTL, including dirigent (DIR) proteins involved in lignification during biotic stress, homologs for innate plant defenses, and homologs of oxidative stress protection. Presence of *qtlpc* and underlying genes contributes *Phytophthora* crown rot resistance through anti-oxidative defense and structural reinforcement to inhibit pathogen penetration and cell death. Indeed, UF-PhytoR1 and similarly resistant $F_{2:3}$ plants remained healthy or exhibited minimal localized lesions around the crown. At least five SNPs lie within these candidate genes (LOD 2.5 to 4.20) offering opportunity for application in marker-assisted backcrossing within a squash breeding program.

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—Scientific Note—

Evaluation of Biostimulant Products for Tomato Production In Florida

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Additional index words. humic acid, mycorrhiza, seaweed extract, *Solanum lycopersicum*

Florida is the largest producer of fresh tomatoes (*Solanum lycopersicum* L.) in the United States. Many nutrient management approaches to improving yield and quality of tomato have been discovered, one of which is the application of biostimulants. Biostimulants are substances or microorganisms that could be applied to the plants and stimulate natural processes to enhance nutrient uptake, stress tolerance, and in some cases, quality attributes, such as nutritional content and shelf life. Some common types of biostimulants include humic acid, fulvic acid, amino acid, crab shell powder, seaweed extracts, algae, and mycorrhiza. To evaluate the effectiveness of various biostimulant products in tomato production in Florida, two independent field experiments were conducted at the Gulf Coast Research and Education Center in Balm, FL.

In the first field experiment, humic acid and seaweed extract products were applied through drenching and drip tape injection, following the manufacturer's recommendations. Both humic acid and seaweed extract treatments significantly increased marketable fruit yields by 10% (92.6 vs. 101.5–101.6 $t \cdot ha^{-1}$) compared to the control, although no significant treatment effects were found on plant canopy area and plant biomass. In the second field experiment, tomato seedlings were treated with a mycorrhizal product via spray application before transplanting or drench application immediately after transplanting. Light microscopy revealed that spray and drench mycorrhizal treatments increased the mycorrhizal colonization rate at harvest from 5.8% to 11.4% and 27.8%, respectively. Spray and drench mycorrhizal treatments also increased marketable fruit yields by 3% and 10% (82.2 vs. 85.0–90.4 $t \cdot ha^{-1}$), respectively, although no significant treatment effects were found on plant canopy area and plant biomass. These results demonstrate the potential of various biostimulant products to improve tomato production in Florida.

Future studies will evaluate treatment effects on root responses in an effort to explain the underlying mechanisms of growth stimulation by various biostumulants. In addition, considering the decent fruit yield in the control treatments, it might be worthwhile to challenge tomato plants with a stress environment to better display the beneficial effects of biostimulants.

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—Scientific Note—

Rapid Screening for Phosphorous-efficient Genotypes from Tomato Germplasm Banks In an Artificial Soil Solution

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Additional index words. tomato, fertilizer, vegetable crop

Tomato is the second most important vegetable crop (potato ranks first) in the world. The total world production of tomatoes is 182.3 million tons with a value of $74.1 billion (FAO, 2019). In USA, the area planted to tomato was 282,000 acres and produced 239,399,500 cwt with a value of $1.6 billion in 2019 (USDA-NASS, 2019). Florida ranked No. 1 in the nation for commercial fresh market tomato production in 2019: planted acreage of 27,000 acres and yield of 7,729,800 cwt with a value of nearly $426 million (USDA-NASS). It is estimated that tomato crops used approximately 3000 tons of P_2O_5. Due to inorganic fixation and low ability of modern cultivars to mobilize sparsely soluble phosphates in soil, up to 80% of phosphorous (P) fertilizers applied may be unused or unavailable to plants (Holford, 1997). This low use efficiency indicates that approximately 2400 tons of P_2O_5 applied do not contribute to tomato productivity every year. This unused P fertilizer may have adverse impacts on the environment. It is imperative for us to improve phosphorus use efficiency for tomato production.

P fertilizer is produced from phosphate rock, which is nonrenewable. This resource is being depleted and may run out soon. Developing P efficient crop varieties is imperative to overcome the predicted P crisis. There is an urgent need to use elite genotypes to breed P-efficient tomato cultivars. Field trials are time and resource consuming and cannot be adopted for easy and rapid screening for P-efficient genotypes. In the present study, a simulated soil solution (SSS) was designed and evaluated to identify P-efficient genotypes of tomato (*Solanum lycopersicum*). A soil solution has a low but buffered concentration of bioavailable P. When a sparely soluble phosphate is used as the only P source in a nutrient solution with extra companion cation, e.g., calcium ion for tricalcium phosphate [$Ca_3(PO_4)_2$] in the following chemical equilibrium, an artificial or stimulated soil solution can be prepared.

Our new publication on tomato has the detailed description for the novel identification method (Bera et al., 2019).

In this study, 30 randomly selected tomato genotypes from commercially available sources were used in hydroponics with tricalcium phosphate as the only P source plus 15 extra mM Ca^{2+} as low-P stress compared with the sufficient P growth conditions with sodium dihydrogen phosphate (NaH_2PO_4) as the control. Specific biomass of shoots (SBS) was used as the criterion to identify the genotypes.

The results showed that 'Japanese Black Trifele Og' (JBT) and 'Great White' Og (GW) were P efficient (SB > 0.5 g/plant) whereas 'Green Tiger' (GT) and 'Mariana' F1 P-inefficient (SB < 0.1 g/plant). JBT had the greatest root morphological traits including root dry mass, length, volume, and fork number. By contrast, the P-inefficient genotypes performed poorly under low-P stress.

Hydroponic identification is a rapid and economical method for screening P-efficient genotypes from germplasm banks and commercially available sources. After the P-efficient genotypes have been identified, they should be planted in on-farm trials to double check their P-use efficiency. Once P-use efficiency is confirmed in on-farm trials, these elite genotypes can be directly used for tomato production with less P input for sustainable vegetable production. Elite genotypes can also be used for P-efficient tomato breeding in the future. P-efficient cultivars are necessary for us to overcome the challenges of P-crisis on the horizon.

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—Scientific Note—

Understanding Lettuce Downy Mildew: Pathogen Distribution and Available Host Resistance In Florida

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Additional index words. fungicide, pathogen races

Florida ranks third in the US for lettuce (*Lactuca sativa* L.) production, with most concentrated on the Everglades Agricultural Area's (EAA) rich "muck" soils. Lettuce Downy Mildew (LDM), caused by *Bremia lactucae*, is the most important lettuce disease worldwide, causing significant direct and indirect yield losses. It is worse with high humidity (> 95%) and temperatures ranging from 40 to 60° F. Fungicide use and resistant cultivars are the most common methods of LDM management (Santos et al., 2018). However, to preserve the stability and efficacy of these control methods, it is essential to understand pathogen races. *Bremia lactucae* is genetically diverse, with 10 races described in the western US and 15 in the European Union. Information regarding pathogenic *B. lactucae* races in Florida remains unknown (van Treuren et al., 2013; Spring et al., 2018). Thus, the first objective of the present work was to identify the races of *B. lactucae* currently present in Florida. The second objective to screen adapted lettuce cultivars and breeding lines for LDM.

For the first objective, diseased samples were collected from several lettuce fields in the EAA. Additionally, a standard set of 17 differential cultivars: 'Green Towers', 'Dandie', R4T57D, UCDM14, 'NunDM15', CGDM16, 'Colorado', FrRsal-1, 'Argelès', 'Muraires', 'Silvinas', 'Bedford', 'Balesta', 'Bartoli', 'Design', 'Kibrille', and 'Bataille' (IBEB, 2020) were planted in two field experiments to collect and characterize races of *B. lactucae*. Diseased samples from random fields and from the differential experiments were used to inoculate 7-day-old seedlings of the universal susceptible cultivar 'Green Tower' grown in Magenta Boxes® and kept in a growth chamber with 12 h light/12 h dark photoperiod at 60 °F. To elucidate races of *B. lactucae* present in Florida, lettuce differentials were evaluated using a subjective rating scale from 0 (no symptoms) to 5 (> 75% symptoms). Ratings were performed 51 and 65 days after planting, in experiments 1 and 2, respectively. A second objective was to evaluate 10 commercial cultivars and 70 University of Florida/IFAS (UF/IFAS) lettuce breeding lines for LDM resistance under natural field conditions.

For objective 1, only 2 isolates were recovered from 200 inoculations during the 2019–20 season. Prophylactic fungicide applications for LDM are very common in lettuce and it is likely that samples collected from grower fields were treated with fungicides before they reached the lab. However, disease severity evaluated on the 17 differentials cultivars indicated that R4T57D, NunDM15, 'Argeles', 'Muraires', 'Silvinas', 'Balesta', and 'Bartoli' showed a resistant reaction to the pathogen. The remaining differentials had varying levels of disease severity with 'Green Towers' consistently highly diseased. These data indicate the presence of a mixture of races 7, 8, and 9 in the field.

In objective 2, commercial lettuce cultivars exhibited high disease severity indicating that all commercial cultivars planted in the EAA are susceptible to LDM. Most UF/IFAS breeding lines were more susceptible than commercial cultivars; however, a few breeding lines had good resistance to field isolates of *B. lactucae* and these may be useful as parents to improve resistance in lettuce against LDM. Additional studies are needed to fully determine the complexity of races of LDM present in Florida, as the pathogen continues to adapt, evolving into newer races. Once a race structure is identified, a detailed screening of the UF/IFAS lettuce germplasm should be conducted.

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—Scientific Note—

Screening for Resistance to *Stemphylium* In Florida-adapted Baby Leaf Spinach

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Additional index words. *Stemphylium* leaf spot

Spinach can be grown either for processing or the fresh market, however, consumers of fresh market demand a convenient, healthy and visually appealing product. Planting density for for baby leaf spinach it ranges from 3.7–7.4 million seeds/acre. Due to this higher planting density, baby spinach is more susceptible to biotic stresses such as diseases. In the Everglades Agricultural Area (EAA) of south Florida, spinach is planted for baby leaf production. Given Florida's warm temperatures and high humidity, Stemphylium leaf spot (SLS), incited by *Stemphylium* species, is an serious disease which causes spots on the leaves; only a 3% incidence of leaf spots is tolerated by retailers. Although fungicides are utilized to manage the disease, there are relatively few products labeled for Florida (Raid and Kucharek, 2006) and the pathogen has already developed insensitivity to popular fungicides. With host-plant resistance a long-term goal for Florida growers, we performed studies to screen spinach accessions for their resistance to SLS and assessed different fungicides.

For the first objective, two field experiments were conducted with 28 and 31 commercial hybrids and experimental accessions during the 2019–20 growing season using a randomized complete design (RCBD). Baby leaf spinach was planted in the field and grown using standard production practices in 9.4-m sections on 1.8-m wide raised beds. Baby spinach was inoculated when plants had four to six true leaves using a mixture of three pathogenic isolates. An additional trial was conducted to screen 26 experimental hybrids in the greenhouse using seedling trays to mimic baby leaf production. 'Kolibri' seeds known to be infested with *Stemphyllium* spp. were planted in every other row to ensure exposure to the pathogen. Disease severity was assessed 16 d following inoculation, and was rated as follows: 0 (no foliar symptoms) to 5 (more than of 75% of the total foliage infected with brown spots).

For accomplish the second objective, two additional field trials were chemically treated with 15 fungicides, using the susceptible 'Stanton'. Treatments were arranged in a RCBD with three replicates.

Baby leaf spinach had different levels of disease pressure in the three germplasm trials. Significant differences ($P < 0.0001$) were found among accessions in both fields experiments and in the greenhouse test ($P = 0.0079$). No genetic material tested was completely immune, but the commercial hybrid 'Caladonia', and experimental accessions SVVC5663, SV2141VS, and SVVC5761 showed the least amount of disease in all three experiments. Many of the experimental accessions had previously shown resistance to *S. boytriosum*, but these experiments were inoculated with *S. versicarum*. This may explain why some of these accessions were not as resistant as expected. Little is known about the race structure of *Stemphylium* recovered in Florida. A pathogen characterization should be pursued to understand pathogen population within the state. Host resistance is not sufficient to combat this disease and growers need efficacious fungicides to design an integrated disease management.

In a trial examining low-risk fungicide treatments, LifeGard, Silmatrix, LifeGard alternated with Cueva, Kocide 3000, and OSO demonstrated significant ($P > 0.0001$) control of SLS but not at commercially acceptable levels. In the second trial, fungicides Luna Sensation, Miravis, Miravis Prime, Xemium, and Pristine significantly ($P > 0.0001$) reduced SLS in baby spinach. While these newer labeled fungicides offered excellent control of leaf spots in baby spinach, disease reduction was not complete. A combination of several compounds and the use of resistant genetics may alleviate the effects of SLS in the long term.

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—Scientific Note—

Varieties of Tomato Saladette Type: Plastic Mulches, Nutrition, and Production In a Greenhouse

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Tomato (*Solanum lycopersicum*) is one of the world's most commonly grown vegetables and is widely consumed. Mexico is one of the leading tomato suppliers, with a 25% market share worldwide. Therefore, it is necessary to continue evaluating and developing varieties with desirable characteristics for the consumer. In this study we evaluated saladette tomato varieties for their market potential. They were grown in soil in a greenhouse with plastic mulch.

Materials and Methods

The current study was carried out for the production, evaluation and subsequent release of saladette-type tomato varieties, by obtaining the best values in the evaluated parameters. The planes evaluated were tomatoes of the varieties V1, V2, V3, V4, V5, V6, V7, V8, V9, and V10. Before carrying out the experiment, three soil samples were taken for subsequent analysis. The experiment was carried out under soil conditions, under a plastic padding. The evaluated variables corresponded to SPAD readings and stem diameter (DT) (mm) at three heights: basal; medial and apex. In addition, the number of bunches was counted. The microenvironment conditions for the tomato variables were: average CO_2 concentration of 280 ppm, air temperature 88 degrees Fahrenheit, and relative humidity 50%. Data were analyzed using SAS software ver. 9.3. An analysis of variance (Proc GLM) was performed. The comparison of means was carried out using the Tukey test with a significance value of 95%.

Results and Discussion

Under an analysis of SPAD values, stem diameter and number of clusters showed highly significant differences ($Pr < 0.0001$, $Pr < 0.0004$ and $Pr < 0.0001$). Moreover, these were corroborated by the coefficient of variation (C.V) 7,591, 23.25, and 17.37, respectively . V9 was the variety whose SPAD readings resulted in the highest values. In the case of stem diameter, the variety with the best results was V6. 'Rafaelo' had the highest number of clusters. In general, the results suggest the best results for those tomato varieties that have important qualities regarding the number of clusters, so it is suggested that we continue evaluating these materials under different conditions such as hydroponics.

Project: Nutritional diagnosis in fruit trees, vegetables, grasses. To El Colegio de Michoacán and LADiPA Laboratory.

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Effect of 'Tahiti' Lime Fruit Size on the Percent of Juice Content Measured by the Standard USDA Volume Method and an Alternative Weight Method

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The percent juice (% juice) content of 'Tahiti' limes is a major factor of fruit quality; the other is fruit size. 'Tahiti' limes with low juice content are of poor quality and a problem in the retail marketplace. Florida's lime industry was once under a federal-state marketing order (MO) which was primarily designed to regulate lime fruit quality and avoid offering poor quality i.e., low juice content limes which depress market demand. The MO stated limes must have a minimum diameter of 1 7/8-inch diameter and at least 42% juice either by volume or weight to be legally sold. Part of the lime fruit quality MO protocol was to determine % fruit juice content by either the volume or weight method; the industry traditionally used the volume method. Experiments were designed to determine the effect of determining % juice content by volume or weight basis and to determine the effect of sampling from mixed lots of limes of different sized fruits versus lots of fruit sized into specific ranges. Percent juice content was determined by volume and weight methods. Percent juice content determined by the weight method was significantly greater than the volume method but had more variability. Sampling limes from a mixed lot of limes resulted in higher % juice content value when two to four limes were undersized and had a lower juice content than large and extra-large limes.

Until 2001, Florida had approximately 3500 acres of 'Tahiti' lime and supplied approximately 50% of all the seedless limes consumed in the U.S. However, due to the citrus canker eradication program the trees were destroyed in an effort to stop the spread of *Xanthomonas citri* the bacterial agent that causes citrus canker in orange and grapefruit trees in central and north Florida. Today, there are only about 60 acres of 'Tahiti' limes grown in Florida, although the potential and interest in re-establishing a 'Tahiti' lime industry in Florida is strong (Evans et al., 2014).

In 1955, the Florida lime industry with the assistance of the USDA-Agricultural Marketing Service and Florida Department of Agriculture and Consumer Services formed a Federal–State Marketing Order (MO) for 'Tahiti' (Persian) limes (Fed-Mkt, 2020). The USDA and industry under the MO formed a Lime Administrative Committee (LAC) made up of growers, packers, and handlers. The purpose of this MO was to organize the industry to regulate the quality (e.g., % juice content and fruit size) and handling (e.g., box dimensions and inspections) of limes grown in and marketed from Florida.

Part of the MO regulations stipulated that the fruit size (diameter) and % juice content required of limes at harvest had to meet science-based and industry agreed-upon standards for high quality limes (Campbell, 1984; Lincoln, 1951; Lynch, 1939; Reeder and Hatton, 1967). However, the MO did not stipulate whether the % juice content had to be determined by volume or weight.

Earlier investigations reported the size (i.e., weight and diameter) and % juice content of 'Tahiti' limes varied with fruit age (maturity), rootstock, and season (Lincoln, 1949; Lynch, 1939; Lynch, 1942; Hatton et al., 1967). Sampling studies showed that there was significant variation in fruit weight, diameter, and % juice content among lime fruit of the same age (Lynch, 1942; Lincoln, 1949). However, there was a trend for the % juice content, fruit diameter and weight to increase with fruit age.

Research to test and correlate various lime fruit attributes such as fruit weight, diameter, % juice content, peel color, acid content, soluble solids, peel thickness, specific gravity, and peel weight were largely unsuccessful (Hatten et al., 1983; Lynch, 1939; Lincoln, 1949). This may have been due to the effect of various environmental (e.g., temperature, soil moisture content, and vapor pressure deficit), biological (e.g., rootstock, hormones) and cultural factors (e.g., fertilizer and irrigation practices) on lime production and quality. Hatton et al. (1983) found a significant positive correlation ($r = 0.98$; 5% level) between lime fruit diameter and weight but poor correlation among % juice content and fruit diameter ($r = 0.31$) or weight ($r = 0.40$). The volume of the fruit was not reported. A low correlation ($r = 0.44$) was also calculated from the fruit weight and % juice content data of Lynch (1939) (J.H. Crane, personal observation).

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Variation in the % juice content among limes of the same size may vary by as much as 15% (Lincoln, 1949). However, some data suggest there was a trend for very large limes (> 2.5-inch dia., "jumbo") to have a higher average % juice content than individual samples of large, medium, and small limes. Random samples that included small, medium and large limes appeared to favor a higher mean juice percentage than samples of individual sizes.

Poor quality 'Tahiti' limes (< 1 7/8-inch dia. and 42% juice) depress repeat sales. For this reason, the definition of legal fruit maturity in limes was set so that the minimum diameter had to be ≥1 7/8 inch (4.76 mm) and contain 42% juice (USDA-II, 1965; USDA-FPB, 1958). The standard method to determine the % juice content was the volume method. First, a random selection of 10 limes per "lot" would be taken. A lot may consist of limes of a particular fruit diameter category (range) or consist of a wide range or mix of fruit with many different diameters from the same grove and harvest date. The volume of the combined ten-fruit sample was then determined by water displacement followed by measuring the juice extracted from said limes (USDA-II, 1965; CFR, 1955).

The sampling protocol prior to picking and prior to packing should be reliable and based on science-based information. The Board of Directors for the LAC were interested in knowing if: 1) there should be a separate % juice standard for limes of different size categories (e.g., small, medium, large, extra-large); 2) if the % juice content of 'Tahiti' limes using the standard volume method and an alternative weight method differed and; 3) the effect of selecting limes from a lot consisting of a particular fruit size category compared to a lot consisting of a mix of different sized fruit.

Materials and Methods

Comparison of volume and weight methods. 'Tahiti' limes were harvested over three seasons. Five 'Tahiti' lime groves were sampled from 20 Feb. through 7 Apr. 1998 (1145 limes harvested), 5 Apr. to 13 May 1999 (1440 limes harvested), and 2 Feb. to 25 Apr. 2000 (4430 limes harvested). All fruit were categorized by fruit diameter, then analyzed as a random sample of 10 whole fruit for volume, 10 fruit for juice volume, and 10 fruit for juice weight (Table 1). Prior to fruit measurements, all fruit were held at ambient temperatures in the shade for 48 h. This was a typical holding period used by packinghouses in an effort to identify and prevent packing of limes which have developed stylar-end breakdown after harvest (Davenport, et al., 1976).

Table 1. Four size categories (ranges) of limes used to determine significance of differences in sample make up and percent juice content.

Tahiti limes sampled from 20 Feb.–7 Apr. 1998		
	Diameter ranges tested	
Size category[z]	(inches)	(cm)
Small	< 1 7/8	< 4.76
Medium	1 7/8–2	4.76–5.08
Large	2–2 ½	5.08–6.35
Extra-large	> 2	> 5.08
Tahiti limes sampled 5 Apr.–13 May 1999 and 2 Feb–25 Apr. 2000		
	Diameter ranges tested	
Size category[z]	(inches)	(cm)
Small	1 3/4–1 7/8	4.5–4.8
Medium	1 7/8–2	4.8–5.1
Large	2–2 1/4	5.1–5.7
Extra-large	2 1/4–2 1/2	5.7–6.4

[z]Legally mature limes must be at least 1 7/8-inch in diameter.

Table 2. Diameter categories of 'Tahiti' limes used to determine the effect of mixed size samples on the percent juice content.[z]

Size categories	Tahiti lime fruit (inches)	Diameter ranges tested (cm)
Small (1)	1 3/4–1 7/8	4.5–4.8
Medium (2)	1 7/8–2	4.8–5.1
Large (3)	2–2 1/4	5.1–5.7
Extra-large (4)	2 1/4–2 1/2	5.7–6.4

[z]Legally mature limes must be at least 1 7/8 inch in diameter.

Repeated 10-lime samples were analyzed. Ten limes were randomly selected from within each category and analyzed separately. The volume of each 10-fruit sample was determined by USDA standard displacement method (USDA-II, 1965). Juice was extracted using a Hamilton Beach Commercial Juice Extractor (Model 932) and the volume of juice recorded. All fruit and juice weights were recorded using a platform balance (Ohaus, Model C305, Parsippany, NJ). The % juice content was calculated on a 10-fruit juice volume or weight basis. The % juice content was calculated as:

% juice content by volume = juice volume (mL)/fruit volume (mL) × 100

% juice content by weight = juice weight (g)/fruit weight (g) × 100

Data were analyzed by one-way ANOVA and means compared by pairwise t-tests of fruit size classes. The variability of the % juice content determined by the volume and weight methods were compared by Pitman's Test.

Effect of fruit size. Data from the 1145 limes harvested from 20 Feb. through 7 Apr. 1998 and the 1080 limes harvested from 17 May to 15 June 1999 were sampled from local groves in Homestead, FL, and held for 48-h prior to analysis. Limes were then categorized by diameter according to four size ranges with the small category range being below the acceptable standard (Table 2). The volume of each individual fruit was determined using the USDA standard displacement method (USDA-II, 1965). Juice was extracted as above, and the volume of juice recorded. Mixed samples consisted of zero to four limes randomly selected from each size category. All fruit and juice weights were recorded. A computer program was used to select 100,000 random iterations of 10-lime samples from within each fruit size category and in 10-lime samples across size categories in spe-

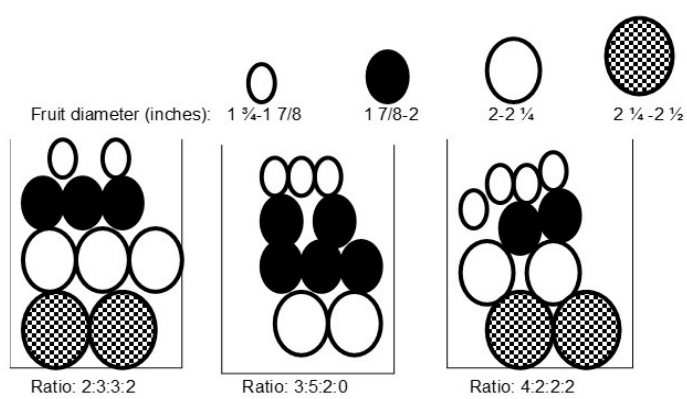

Fig. 1. Visual depiction of lime fruit diameter sizes used to test the effect of various ratios (numbers) of different size limes on the percent juice content.

Table 3. 'Tahiti' lime percent juice content determined by the volume and weight methods for four size categories.[z]

Tahiti limes harvested	Tahiti limes fruit dia. size categories (inches)	Mean percent juice content (by vol.)	Mean percent juice content (by wt)
20 Feb.–7 Apr. 1998	Small, < 1 7/8	20.1 (± 6.0)	25.4 (± 7.4)
	Medium, 1 7/8–2	24.9 (± 5.1)	30.2 (± 6.1)
	Large, 2–2 ½	30.2 (± 5.7)	35.3 (± 6.3)
	Extra-large, > 2	37.0 (± 7.4)	41.5 (± 8.3)
5 Apr.–13 May 1999	Medium, 1 7/8–2	40.0 (± 2.9)	42.5 (± 2.6)
	Large, 2–2 1/4	38.4 (± 1.7)	40.3 (± 1.8)
	Extra-large, 2 1/4–2 1/2	49.1 (± 2.1)	51.2 (± 2.1)
2 Feb.–25 Apr. 2000	Small, 13/4–1 7/8	38.4 (± 2.7)	40.7 (± 2.8)
	Medium, 1 7/8–2	40.9 (± 3.5)	42.1 (± 7.4)
	Large, 2–2 1/4	43.3 (± 3.4)	45.2 (± 3.1)
	Extra-large, 2 1/4–2 1/2	45.4 (± 3.1)	45.4 (± 3.0)

[z]A significant difference was found in percent juice content among the four size categories tested by the volume or the weight method (*t*-test; $P \leq 0.95$ confidence).

cific size ratios (called a mixed sample) (Fig. 1). The data were analyzed by one-way ANOVA and means separated by pair-wise *t*-tests.

Results and Discussion

Comparison of volume and weight methods. 'Tahiti' lime juice content from the 1998 harvest was below the 42% minimum standard for both methods (Table 3). During this sampling period there were two 18-d periods with little to no rainfall (FAWN, 2020). Furthermore, limes harvested in late winter-early spring generally have less juice content than those harvested in summer (Lincoln, 1939). By contrast, almost all limes harvested in 1999 and 2000 had > 42% juice content. A highly significant ($P < 0.0001$) difference was found for the % juice content among fruit size (diameter) categories determined by the volume or weight methods (Table 3). The % juice content of fruits increased with increasing fruit diameter whether measured by the standard USDA volume method or the weight method (Table 3).

In general, the % juice content determined by the weight method significantly ($P < 0.0001$) exceeded the % juice content determined by the volume method (Table 3). However, Pitman's Test found the % juice content determinations by the weight method were significantly ($P < 0.0001$) more variable than those by the volume method.

Effect of fruit size. The % juice content of limes selected from mixed lots was significantly higher than from limes selected from small, medium, large and extra-large categories (Table 4). On average, the % juice content obtained from a random sampling of ten limes from a mixed lot exceeded the % juice content of ten limes selected from a lot of only small- (< 1 7/8 inch) or medium-sized (1 7/8–2 inch) limes by 8.27% and 3.40%, respectively (Table 5). By contrast, the % juice content measured from a random sampling of a mixed lot had on average, 1.90% and 8.65% lower % juice content than from limes selected from a lot of either large or extra-large sizes respectively (Table 5).

Interestingly, the % juice content of mixed samples from the specific ratios of different diameters tested all resulted in 46% juice content or higher (Table 4). This suggests that the fruit volume and juice content of medium limes and large or extra-large limes compensated for two to four small limes (1 ¾ to 1 7/8-inch dia.) in a mixed sample. Therefore, from 20 to 40% of the limes in a lot that is tested using a mixed sample (which includes two to four undersized limes <1 7/8 inch dia.) may appear to meet Florida lime quality standards (i.e., 42% juice content) when in fact it does not (Table 6).

Table 4. Effect of mixing various 'Tahiti' limes of different size diameters on the percent juice content by the standard USDA volume method and weight method.

Lime sample mixing status	Size of limes in each sample	Number of limes in category 1, 2, 3, and 4 in each sample[y]	Mean percent juice content (%±SD)[z] USDA volume method	Mean percent juice content (%±SD)[z] Weight method
Non-mixed lots	Small, 1 3/4 – 1 7/8	10:0:0:0	38.4 ± 2.7	40.7 ±2.8
	Medium, 1 7/8 – 2	0:10:0:0	40.9 ± 3.5	42.1 ±7.4
	Large, 2 – 2 1/4	0:0:10:0	43.3 ± 3.4	45.2 ±3.1
	Extra-large, 2 1/4 – 2 1/2	0:0:0:10	45.4 ± 3.1	45.4 ±3.0
Lime sample mixing status	Size of limes in each sample	Ratio of limes of each size	USDA volume method	Weight method
Mixed lots	Small, medium, large, and extra-large	2:3:3:2	48.1 ± 1.2	50.3 ±1.3
		3:5:2:0	46.8 ± 1.0	49.0 ±1.0
		4:2:2:2	46.9 ± 1.2	49.1 ±1.1

[z]A significant difference was found in percent juice content among the four size categories tested by the volume or the weight method (*t*-test; $P \leq 0.95$ confidence).
[y]A total of 10 limes were in each sample.

Table 5. Effect of sampling ten limes randomly from a lot of limes of mixed sizes compared to selecting 10 limes randomly from a specific size range.

Tahiti lime dia. range categories (inches)	Percent juice content results	
	Random selection from a lot of mixed lime sizes	Random selection from a lot of limes within a dia. size range
small, < 1 7/8	8.27% greater reading	
medium, 1 7/8–2	3.40% greater reading	
large, 2–2 1/2		1.90% greater reading
extra-large, > 2		8.65% greater reading

Table 6. The percentage of undersized (illegal) limes that pass the legal percent juice content (42%; USDA volume method) when part of a mixed sample in the ratios tested.

Ratio of limes of category 1, 2, 3, and 4	Percent juice content USDA method (%)	Percent of size category 1 (undersized) limes in the sample (%)[z]
2:3:3:2	48.1 ± 1.2	20
3:5:2:0	46.8 ± 1.0	30
4:2:2:2	46.9 ± 1.2	40

[z]Limes of 1 3/4–1 7/8-inch diameter.

Conclusion

In general, the % juice content increased with fruit size whether determined by either the volume or the weight method. The % juice content by the weight method resulted in slightly (although significantly) higher % juice content values compared to those by the volume method. This suggests that requiring different standards of juice content by fruit diameter may be of some value, although if the required 42% juice content standard is exceeded this may not be necessary. However, the % juice content measurements by the weight method were more variable than by the volume method. With respect to reliability, measuring the % juice content by the weight method does not appear to have any advantage over the volume method.

When ten limes were selected at random from a mixed lot that included undersized limes (small, 1 3/4–1 7/8 inch dia.) were compared to those selected from a particular size category of legally minimally sized (≥ 1 7/8 inch dia.) or greater dia. of limes, the average % juice content of was affected by the proportion of small, medium, large, and extra-large limes in the lot. Sampling from a mixed lot of fruit which resulted in higher % juice content masked the fact that up to 40% of the limes in the lot could have < 42% juice. This finding suggests that the previous practice of selecting limes at random from a mixed lot of fruit may have biased the % juice content determination in favor of higher readings for the lot. This made it appear that all the limes met the 42% juice content standard when in fact, from 20 to 40% may have had a lower juice content.

Perhaps producers and packers should randomly sample limes for diameter prior to harvesting and/or packing to be sure that the vast majority of fruit diameter of the lot meets or exceeds 1 7/8-inch and, if it passes this standard, then test for the ≥ 42% % juice content requirement.

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—Scientific Note—

Produce Safety In Florida: Education, Outreach, and Compliance

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The Food Safety Modernization Act (FSMA) was signed into law in 2011 and granted the US Food and Drug Administration (FDA) authority to create seven new foundational food safety rules including the Produce Safety Rule (PSR). For the first time, FSMA PSR establishes science based minimum standards for the safe growing, harvesting, packing, and holding of produce. The primary focus of PSR was to reduce foodborne illness/outbreaks through requirements that mitigate food safety risk by preventing microbial contamination related to: worker training, health, and hygiene; soil amendments; wildlife/domesticated animals; agricultural water quality; sanitation; and recordkeeping. This has established a demand for food safety education and outreach activities in Florida to help farms meet the standards of the PSR. The estimated number of farm operations in Florida covered under the PSR is 3392; implementing the PSR requirements on all each diversified farm represents a challenge.

To address these challenges in Florida, the University of Florida/IFAS (UF/IFAS) Food Safety Team has been building capacity to provide outreach and technical assistance. The team offers a number of resources to assist Florida producers implement the PSR. Some of the key resources are Produce Safety Alliance (PSA) Grower Trainings and conducting voluntary visits called "On Farm Readiness Reviews" (OFRRs) to help interested farmers assess their readiness for PSR regulatory inspections and apply the PSR on their individual operations. Trainings are conducted using a standardized curriculum developed by the Produce Safety Alliance whereas OFFR's are conducted based on guidelines and materials developed by National Association of State Departments of Agriculture. One of the main goals of these activities is to improve food safety knowledge and prepare growers for PSR inspections.

In order to deliver training materials for PSA training, the UF/IFAS faculty and extension associate team consists of 53 trainers or lead trainers of the PSA training curriculum. Trainers from Florida Department of Agricultural and Consumer Services (FDACS), Florida A&M, Publix (supermarket), and private consultants have also been developed and added to the team, resulting in 108 trainers.

Since 2016, UF/IFAS has offered 72 PSA Grower Trainings and beginning 2018, conducted 50 OFRRs collaborating with FDACS. In 2020, faced with new challenges presented by COVID-19, PSA trainings are being offered remotely and OFRR's are conducted based on CDC guidelines.

To measure food safety knowledge gain during PSA trainings, pre- and post-assessments, developed by the Southern Center, were given to each attendee. From 2016–19, out of 25 points, participants scored an average of 16.46 on the pre-test and 20.26 on the post-test ($t = -1.72$, $P < 0.05$). Recently pre-test scores have increased to 19.24, indicating an increase in basic knowledge of food safety; however post-test scores remain significantly higher than pre-test scores indicating a significant increase in knowledge.

To assess the OFRR program, at the end of the farm visit, the assessors fill out an anonymous on-line survey to record information about the farms' areas of need in terms of overall readiness for PSR compliance. Post OFRR surveys indicate (n = 43) 46% of farms meet the minimum PSR requirements and only 14% needed significant improvements. Assistance required by the farms to meet PSR requirements are time (39%), technical support (19%), and facility upgrades (17%). The top three areas that required the most improvement are: postharvest sanitation (28%); worker training (26%); and preharvest water (18%).

These programs have facilitated knowledge gain and compliance of Florida farms regarding the PSR. These efforts help ensure that the produce grown in Florida will continue to meet escalating market-driven and regulatory food safety standards.

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—Scientific Note—

Sensory and Quality Analysis of HLB-tolerant Citrus Cultivars for Juice Production

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Additional index words. huanglongbing, orange

Huanglongbing (HLB) is a bacterial disease that has greatly affected the citrus industry since it was first found in the United States in 2005. Since then, there has been an over 70% decrease in orange production. Current research is investigating ways to reduce the effects of HLB on citrus trees as well as identifying cultivars that are tolerant to HLB. One of the cultivars that appears HLB-tolerant is known as 'Sugar Belle®'. 'Sugar Belle®' is a cross between clementine tangerine and 'Minneola' tangelo and is recognized as one of the most HLB-tolerant commercial cultivars currently grown. This study aims to evaluate the sensory and chemical analysis of juice and juice blends of 'Sugar Belle®' and common sweet oranges (*Citrus sinensis*) used in juice production. The use of 'Sugar Belle' in orange juice will allow for an alternative to the use of HLB susceptible sweet orange cultivars which are more expensive to maintain and produce less.

The effect of 'Sugar Belle®' juice on sweet orange juice was analyzed by performing two separate sensory panels analyzing the sensory attributes of 'Sugar Belle®' with 'Hamlin' and 'Sugar Belle®' with 'Valencia'. Consumer panelists were asked to evaluate five samples: 100% 'Sugar Belle®', 100% sweet orange (either 'Hamlin' or 'Valencia'), 90/10 sweet orange/'Sugar Belle®', 50/50 sweet orange/'Sugar Belle®', and 100% Florida commercial orange juice. These sensory evaluations indicated that in the first panel both the 50/50 blend of 'Hamlin'/'Sugar Belle®' and 90/10 'Hamlin'/'Sugar Belle®' were preferred by consumers in overall liking, flavor liking, and overall appearance. In the second panel 100% 'Valencia', 90/10 'Valencia'/'Sugar Belle®', 50/50 'Valencia'/'Sugar Belle®' all were indicated to have high consumer acceptability in overall liking, flavor liking, and overall appearance. These samples were also ranked higher by consumers compared to other samples presented.

Blending 'Sugar Belle®' with juice from sweet oranges produced a product that had equal to or greater consumer acceptability than 100% sweet oranges juice and a commercial product. An understanding of the chemical makeup will be determined by performing chemical analysis on these juices used in sensory analysis. The future use of 'Sugar Belle®' in orange juice production over time will be validated by repeating this research to account for changes in subsequent harvests.

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—Scientific Note—

Food Safety and Regulatory Guidance for Florida Food Processors Operating Out of Shared-use Commercial Kitchens

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Additional index words. food-based businesses

Shared-use commercial kitchens (e.g. incubators, accelerators) have emerged as popular options for beginning food entrepreneurs to use as they start a food-based business. In Florida, there are currently at least 30 shared-use commercial kitchens operating to provide adequate space and equipment that meets regulatory standards so that food may be processed and sold through retail markets or wholesale distribution (Krug and Ellis, 2020).

While these facilities provide an economically feasible option for clientele to start or grow a food business, there are still several hurdles they may encounter. One challenge food entrepreneurs face is navigating the various food regulations in order to register their businesses and pass initial and subsequent inspections. Florida is the only state with three different agencies who enforce food regulations in some manner [Florida Department of Agriculture and Consumer Services (FDACS), Department of Business and Professional Regulation (DBPR), and Florida Department of Health (FDOH)], which adds to the complexity of navigating these regulations. Another hurdle may be a lack of knowledge in food safety principles and the ability to execute adequate practices in order to meet the requirements of regulations. Previous survey work with food safety educators and regulators also supports the notion that there may be food safety and regulatory knowledge gaps associated with small food businesses (Harrison et al., 2016). Furthermore, the shared-use aspect of these types of facilities means that multiple food business operators may be processing in the same kitchen space, which heightens the importance for adequate sanitation and allergen control to prevent cross-contamination or cross-contact between food products. University of Florida/IFAS (UF/IFAS) extension agents have worked to develop and implement program activities to help food entrepreneurs overcome these hurdles, but it is important to fully understand the needs of the target audience during this process. The objective of this study was to determine clientele needs and help shape current and future extension education activities such as consultations and workshops.

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Surveys were distributed to current and potential members of shared-use commercial kitchens located around southern Florida in 2018–19. Surveys were conducted using the software Qualtrics, kept anonymous, and included an appropriate informed consent statement. Questions focused on gathering information about the target audience related to their past experiences operating a food business or selling a food product, the types of food products and method(s) of sale for current or future food business ventures, and prior food safety trainings attended/experience in the field.

Survey responses (n = 51) indicated that only 23.5% of clientele were familiar with the specific food regulations applicable to the food product they intended to sell, while only 35.3% had received any prior formal food safety training such as Servsafe®. 52.3% had produced a food product for sale in the past, yet none indicated that they had ever created a formal food safety plan for a product. These results affirm the hypothesis that beginning food entrepreneurs who plan to operate out of a shared-use kitchen may lack knowledge of food regulations and food safety. As a result, UF/IFAS Extension faculty developed and modified training activities to increase clientele knowledge in these areas and provide other relevant resources for their food businesses. This included consultations directly with clientele or workshops which used representatives from Florida food regulatory agencies as speakers. These efforts have been successful in connecting clientele with the regulatory agencies, increasing clientele understanding of applicable regulations (e.g. FDA Food Code, Current Good Manufacturing Practices), and enhancing food safety practices in shared-use commercial kitchen facilities.

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—Scientific Note—

Recovery of Ripening by Mature Green Banana Fruit following 1-Methylcyclopropene Application

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Additional index words. color change, respiration, ethylene, internal gas, aqueous 1-MCP application

Ethylene is associated with important postharvest developmental processes including ripening, senescence and overcoming ethylene effects which can be important to extend the shelf-life of climacteric fruits like banana (*Musa acuminata*, AAA group). An example would be avoidance of ripening initiation in transit during international shipments. 1-methylcyclopropene (1-MCP) blocks ethylene receptors, making bananas insensitive to ethylene; however, the treated fruit must eventually recover ripening competency, which can be problematic in banana. Therefore, when developing a 1-MCP treatment protocol for climacteric fruits, it is necessary to determine an appropriate 1-MCP concentration to delay ripening followed by a procedure allowing eventual recovery of normal ripening. In this study, we determined an effective 1-MCP concentration for a 60-s aqueous treatment of banana fruit in order to delay the ripening process. We also investigated the effect of exposure to exogenous ethylene before or after 1-MCP treatment on ripening recovery.

Mature-green (MG) banana fruit were (A) treated with 0 or 100 $\mu L \cdot L^{-1}$ ethylene for 24 h at 20 °C and 95% relative humidity (RH) followed by immersion in 0, 10, 25, or 50 $\mu g \cdot L^{-1}$ 1-MCP (a.i.) solution at 23°C for 60 s then ripened in air at 20 °C and 95% RH or (B) immersed in 0, 50 or 100 $\mu g \cdot L^{-1}$ 1-MCP followed by continuous exposure to 0 or 100 $\mu L \cdot L^{-1}$ ethylene at 20 °C and 95% RH. The rates of respiration and ethylene production, internal CO_2 and ethylene concentrations, and peel color changes and internal appearance were evaluated every other day until the fruit in a given treatment reached ripeness stage 4 (on a 1–7 scale). The fruit were then subjected to daily measurements and ripeness stage evaluations. Data were subjected to repeated measures analysis of variance (RM-ANOVA) using JMP statistical software. Fisher's least significant differences (LSD, $P \leq 0.05$) were determined to compare differences between treatment means following identification of a significant ANOVA effect.

Ripening of MG banana fruit to stage 7 after immersion in 10, 25, oe 50 $\mu g \cdot L^{-1}$ 1-MCP without prior ethylene exposure was significantly delayed (by 2, 10, and 12 d, respectively), but ethylene pre-treatment prevented 1-MCP-induced inhibition of ripening. When MG bananas were immersed in 0, 50, or 100 $\mu g \cdot L^{-1}$ 1-MCP solution followed by continuous exposure to 0 or 100 $\mu L \cdot L^{-1}$ ethylene at 20 °C, 1-MCP plus ethylene fruit initiated ripening 4 to 6 d earlier than ethylene-free fruit. The time to reach stage 7 for 1-MCP plus ethylene fruit was 2 d shorter than fruit ethylene. These 1-MCP-induced delays in ripening were observed for subjective and objective determinations of color development, onset of climacteric rises in respiration and ethylene production, and for internal CO_2 and ethylene concentrations. Uneven recovery from 1-MCP was observed for fruit at stage 5, which had a softer central pericarp and firmer external pericarp that adhered to the peel. This was only partly prevented by exposure to ethylene after 1-MCP (Fig. 1). In summary, 25 $\mu g \cdot L^{-1}$ was the minimum effective 1-MCP concentration for 60-s immersion of MG bananas to delay ripening at 20 °C, providing a 10-d extension over untreated fruit. Prior exposure of banana fruit to ethylene largely negated the effect of 1-MCP on ripening. Post-1-MCP treatment ethylene exposure accelerated the recovery of ripening, which suggests that ethylene may promote generation of new ethylene receptors. Our results for pre- and post-1-MCP ethylene treatments agree with previous reports showing the effects of exogenous ethylene treatments in simultaneously promoting ripening and mitigating 1-MCP effects. To ensure uniform ripening of banana following 1-MCP treatment, the continuous presence of a saturating dose of ethylene is essential.

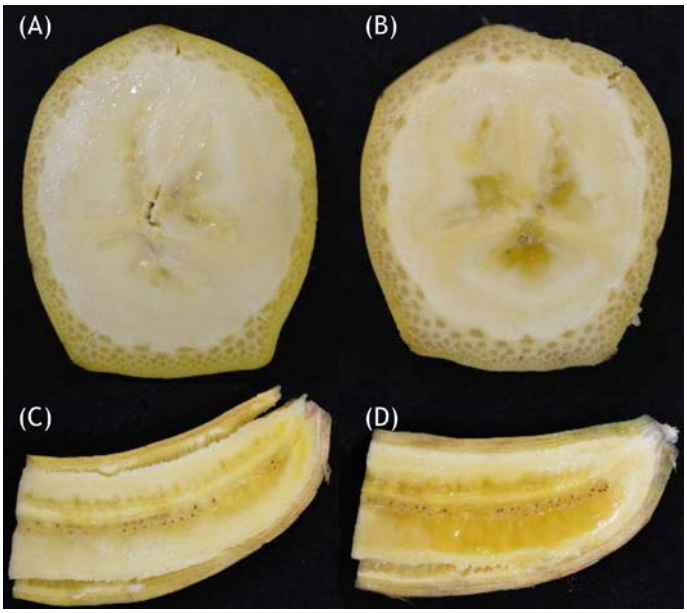

Fig. 1. Pericarp softening shown in cross section (**A, B**) and longitudinal section (**C, D**). (**A, C**) normally ripe banana (-ETH) on day 13; (**B, D**) banana treated with 100 $\mu g \cdot L^{-1}$ 1-MCP followed by 100 $\mu L \cdot L^{-1}$ ethylene on day 25 (unevenly ripe).

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Nano- and Micro- Carnauba Wax Emulsions versus Shellac Protective Coatings on Postharvest Citrus Quality

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Additional index words. Aroma volatiles, *Citrus reticulata*, *Citrus sinensis*, *Copernica cerifera*, fermentation, mandarin, tangor

Abstract. Coatings are generally applied to fruit as microemulsions, but nanoemulsions are still experimental. 'Nova' mandarins (*Citrus reticulata*) were coated with shellac or carnauba (*Copernica cerifera*) microemulsions or an experimental carnauba nanoemulsion; these were compared with an uncoated control during storage for 7 days at 20 °C. Coatings were also tested on 'Unique' tangors (*C. reticulata* × *C. sinensis*) stored for 14 days at 10 °C followed by a simulated marketing period of 7 days at 20 °C. Fruit quality evaluations included weight loss, gloss, soluble solids (SS), titratable acidity (TA), pH, SS/TA ratio, internal CO_2, O_2, fruit juice ethanol, and other aroma volatile content. Sensory visual shine and tangerine (*C. reticulata*) flavor rank tests after storage were conducted, followed by an off-flavor rating. The carnauba waxes resulted in less weight loss compared with the uncoated control and shellac coating during both experiments. There were no differences in gloss measurements of 'Nova' mandarins; however, shellac-coated fruit ranked highest for shine in a sensory test. For 'Unique' tangors, initially, shellac showed the highest gloss (shine) measurement; however, at the end of storage, the nanoemulsion exhibited the highest gloss, although it was not different from that of the microemulsion. Similarly, after storage, the nanoemulsion ranked highest for visual shine, although it was not different from that of the microemulsion. There were only minor differences in SS, TA, pH, and SS/TA among treatments. The internal CO_2 gas concentration and juice ethanol content generally increased and internal O_2 decreased during storage. The highest levels of CO_2 and ethanol were found for the shellac treatment, as was the lowest O_2, indicating anaerobic respiration. There were only minor differences among the other coating treatments; however, they were only sometimes different from those of the control, which generally had the highest O_2, lowest CO_2, and lowest ethanol. Shellac and the carnauba microemulsion also altered the volatile profile more than the control and the nanoemulsion did, especially for 'Unique' tangors. For 'Unique' tangors, the control and nanoemulsion ranked highest for tangerine flavor and had the least off-flavor at the end of storage. Among the coatings tested, the carnauba emulsions demonstrated less water loss, imparted more sustainable gloss, and caused less ethanol production than shellac, with the nanoemulsion exhibiting higher gloss measurements, less modifications of the atmosphere and volatile profile, and, consequently, better flavor compared with the microemulsion.

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Tangerine/mandarin (*Citrus reticulata*), clementine (*Citrus clementina*), and satsuma (*Citrus unshiu*) world production in 2018–19 comprised 32.0 million tonnes of fresh fruit (U.S. Department of Agriculture, 2020). China is the largest producer of mandarins (22.0 million tonnes) and the second largest producer of fresh oranges (*Citrus* sp.) in the world, at 7.2 million tonnes. The largest sweet orange (*C. sinensis*) producer is Brazil, with a yield of 19.4 million tonnes (Food and Agricultural Organization of the United Nations, 2020). The development and application of protective coatings are recognized as alternative environmentally friendly approaches to reduce losses and improve postharvest conservation (Nayak et al., 2019).

Coatings provide a barrier between fruit and the external environment, induce a modified atmosphere within fruit (decreased O_2 and increased CO_2), and reduce water vapor diffusion,

thus influencing respiration and transpiration rates, respectively. Coatings can influence the aroma volatile profile (Baldwin et al., 1995a, 1995b; El Hadi et al., 2013). For citrus (Rutaceae), the main criteria for coatings are the ability to impart shine, improve sales, and retard water loss to reduce shrinkage while maintaining fresh flavor (Hall, 2012). Resins and waxes are generally used (Bai and Plotto, 2012) because they are hydrophobic to varying degrees and impart shine.

Certain coating formulations can cause anaerobic respiration due to modification of the fruit internal atmosphere, causing fruit to produce high levels of ethanol and acetaldehyde, leading to off-flavor and trapping off-flavors within the fruit (Baldwin et al., 1999). In general, fruit produce a collection of volatile compounds that comprise their characteristic aroma, which is important for acceptability by consumers (El Hadi et al., 2013). Nisperos-Carriedo et al. (1991) reported that citrus, stored for 13 d at 21 °C, showed an increase in some aroma volatiles, including alcohols, which were higher in commercial wax-coated oranges than in uncoated oranges, thus altering the aroma profile. This highlights the need to tailor functional and permeability proprieties of coatings to suit the unique requirements of every fruit and vegetable under specific storage regimes to achieve the best quality.

Coating fruit surfaces serves to replace the natural layer of wax removed by cleaning and handling processes in packing houses. Coatings generally used for citrus include microemulsions containing resins, waxes or blends of shellac, candelilla (*Euphorbia cerifera*) wax, carnauba (*Copernica cerifera*) wax, beeswax (*Apis* sp.), polyethylene, or petroleum waxes (Hall, 2012; Palou et al., 2015). Among commercial citrus coatings, shellac resin has been widely used alone or as a major component of "waxes" (Hagenmaier and Shaw, 1991) due to its ability to impart high shine (often measured as "gloss" by a gloss meter). Shellac does help to maintain moisture and reduce shrinkage, but not to the extent of true waxes. Carnauba wax has been largely used to form conventional microemulsion coatings, alone or mixed with other waxes and resins, to optimize shine (Bai and Plotto, 2012; De Freitas, et al., 2019; Luangtana-Anan and Limmatvapirat, 2019; Palou et al., 2015).

Wax emulsion coating properties, such as size and stability, are partly dependent on the size of the imbedded lipid micelle. Generally, the smaller the lipid micelle, the more clear, shiny, and stable the emulsion. Therefore, most fruit wax emulsions are microemulsions with microsized lipid micelles (typically 0.5 μm) (Hall, 2012). As technology has advanced, nanoemulsions (nano = 1–100 nm) have been developed; however, there are few studies of carnauba nanostructure-based coatings compared with those of polysaccharide-based nanocoatings such as nanochitosan (González-Saucedo et al., 2019; Nguyen and Nguyen, 2020; Pilon et al., 2014). However, Miranda (2015) developed a carnauba wax nanoemulsion coating that exhibited small nano-sized (≈44 nm) lipid micelles, allowed gas exchange, reduced water loss, and imparted shine and stability. These coatings, however, may have different gas permeability properties compared with conventional microemulsions due to the change in structure.

In this context, edible coatings can contribute to fruit quality and shelf life by improving visual quality and reducing postharvest losses due to desiccation if the formulation does not cause off-flavor due to modification of the internal fruit atmosphere. During this study, a carnauba nanoemulsion coating was evaluated and compared with typical shellac and carnauba microemulsion coatings on two citrus species to determine if the nanoemulsion or microemulsion coating structure would impart more shine and affect coating permeability, thus affecting the fruit flavor.

Materials and Methods

Fruit. Approximately 200 'Nova' mandarins (*C. reticulata*) and 300 'Unique' tangors (*C. reticulata* × *C. sinensis*) were used in two experiments. 'Unique', or 'Ortanique', is believed to be a spontaneous hybrid of a sweet orange and a mandarin that was first found in Jamaica (Nugent et al., 1967). Fruit were obtained from Al's Family Farms Citrus, Fort Pierce, FL. The fruit were selected for uniformity of size (≈70 and 80 mm in diameter for 'Nova' and 'Unique', respectively) and lack of defects, washed with commercial fruit cleaner (JBT 395; JBT FoodTech, Lakeland, FL), rinsed, and sanitized with 100 $mg \cdot L^{-1}$ peroxyacetic acid (PAA) (Jet-Oxide; Jet Harvest Solutions, Longwood, FL) for 3 min and then air-dried at room temperature.

Coating preparations. A conventional carnauba wax microemulsion was formulated according to Hagenmaier and Baker (1997), with a slight modification. Carnauba wax emulsion was prepared in an open reactor by heating 45 g of carnauba wax type I (Strahl & Pitsch, West Babylon, NY), 5 g oleic acid, 5 g myristic acid, 28 g ammonium hydroxide (all Sigma-Aldrich Chemical Co., St. Louis, MO), and deionized water (175 g) at 105 °C with constant mechanical stirring (800 rpm) for 3 m. Then, under mechanical stirring, the emulsion was cooled to 50 °C. The carnauba wax nano-emulsion was prepared with an oil phase composed of carnauba wax type 1 (150 g) and oleic acid (30 g) from Sigma-Aldrich Chemical Co. (St. Louis, MO). The water phase was composed of 20 g ammonium hydroxide, dimethylpolysiloxane (0.1 mL; Sigma-Aldrich Chemical Co.), and deionized water (775 g). Formulation of the nanoemulsion was accomplished by the inversion phase of the water in oil (W/O) to oil in water (O/W) system. The diameter size of the lipid micelles obtained was 44 ± 8 nm, with a narrow polydispersion index (0.28) and zeta potential of –43.8 mV (Miranda, 2015) measured by a zeta potential analyzer (Zetasizer Nano ZS; Malvern Instruments, Westborough, MA). The microemulsion, in comparison, had a larger dispersion of droplet sizes and a higher polydispersion index (PDI = 1.0) than the nanoemulsion (PDI = 0.28).

Fruit processing and coating. This research was performed during two experiments. The first experiment tested coatings on 189 'Nova' mandarin fruit with the following treatments: laboratory-made, simulated commercial carnauba wax microemulsion; a commercial shellac microemulsion (Stay Fresh 590HS, JBT FoodTech); an experimental carnauba nanoemulsion wax; and uncoated fruit. The commercial shellac microemulsion was included for a true commercial comparison of shellac resin. The second experiment was conducted with the same treatments but applied to 289 'Unique' tangor fruit. Fruit were coated by spreading 1 mL of coating per fruit by hand while wearing latex gloves. Coated fruit were then dried with a heat gun (50 °C) for 60 s before analyses and storage, simulating industry drying techniques.

During the first experiment, 'Nova' mandarins were stored for 7 d at 20 °C and quality analyses were performed initially (0 d) and at the end of storage (7 d). During the second experiment, 'Unique' fruit were stored at 10 °C for 14 d, followed by a

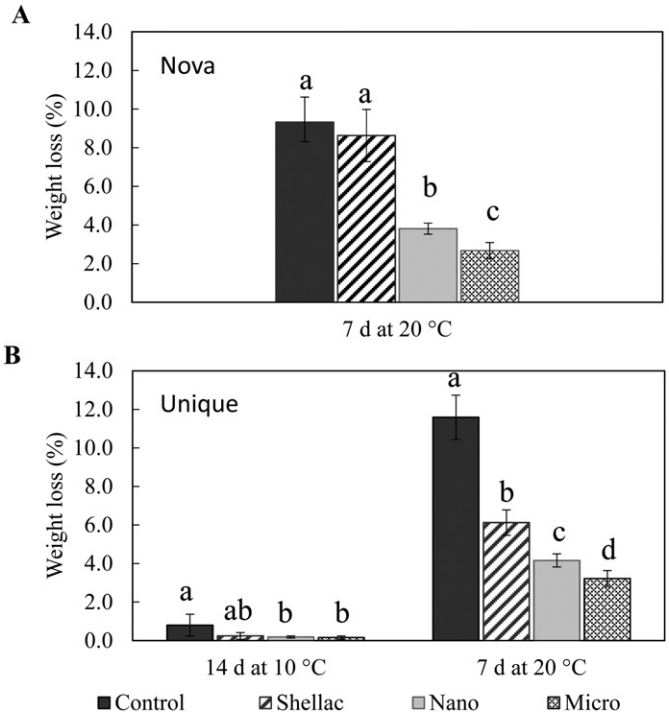

Fig. 1. Nanoemulsions and microemulsions of carnauba wax that help to slow water/weight loss from citrus fruit compared with a commercial shellac microemulsion. (**A**) Weight loss of coated and uncoated 'Nova' mandarins and (**B**) weight loss of 'Unique' tangors (10 fruit/treatment). For each storage period, columns with different letters are significantly different according to Duncan's test ($P < 0.05$).

simulated marketing period of 7 d at 20 °C, which is more typical for the industry. Quality analyses were performed initially (0 d), after 14 d of cold storage (10 °C), and again after the simulated marketing period (7 d at 20 °C). The relative humidity (RH) was 80% to 85% for all storage.

Fruit quality. Fruit were gently hand-juiced using a food service juicer at room temperature to avoid peel oil in the juice (Baldwin et al., 2012). Juice aliquots (3 mL) were immediately pipetted in crimp-capped vials for gas chromatography (GC), frozen in liquid N_2, and stored at –80 °C to optimize the volatile content. In addition, 40 mL of juice was pipetted into plastic tubes and stored at –20 °C for sugar and acid analyses. The SS content of the juice was determined by the refractive index with a digital refractometer (PR-101; Atago, Tokyo, Japan). The TA and pH were calculated from titration of 10 mL of juice with 0.1 $mol \cdot L^{-1}$ NaOH to the pH 8.1 endpoint using an autotitrator (DL50; Mettler Toledo, Columbus, OH) and expressed as the percent of juice. The ratio (SS/TA) was the proportion of sugar and acid (Baldwin et al., 2012). For SS, TA, pH, and SS/TA, 15 fruit were juiced per treatment (five composite replicates of three fruit each).

From this same juice, ethanol and other volatile compounds were quantified by GC (6890; Agilent Technologies, Santa Clara, CA) using a standard curve with authentic standards (Baldwin et al., 2012). Temperatures of the oven, injector, and detector were 70, 250, and 250 °C, respectively. For quantification of ethanol and other aroma volatiles, 3 mL of fruit juice was transferred to a 10-mL crimp-capped vial, rapidly frozen in liquid N_2, and then stored at –80 °C. Frozen samples were later thawed under running tap water and inserted in a Gerstel multipurpose auto sampler for head space injection (3 mL) in the gas chromatograph equipped with a polar column (Stabilwax; Restek Corp., Bellefonte, PA), a nonpolar low-bleed column (HP-5; Agilent Technologies) and a flame ionization detector. The gas flow rates for He, H_2, and air were 10, 35, and 350 $mL \cdot min^{-1}$, respectively. Temperatures of the oven, injector, and detector were 90, 200, and 250 °C, respectively. In addition to ethanol, common citrus volatiles were analyzed, including the alcohols hexanol, *cis*-3-hexenol, *trans*-2-hexenol, methanol, 2-methylpropanol, α-terpineol, and linalool; aldehydes acetaldehyde, decanal, and hexanal; esters methybutanoate, ethylbutanoate, ethylacetate, ethylhexanoate, and ethyl-3-hydroxyhexanoate; and terpenoids valencene, limonene, myrcene, α-pinene, sabinene, and γ-terpinene. Volatile compound identification was confirmed using solid phase microextraction (SPME) (50/30 μm DVB/CAR/PDMS; Supelco, Bellefonte, PA) and GC-mass spectrometry (MS), as reported by Wang et al. (2015). The instrument and column for SPME injection were performed using GC-MS (6890 GC + 5973N MS, Agilent Technologies) with a nonpolar column (0.25 mm × 60 m, 0.50-μm-thick film thickness, DB-5, Agilent Technologies).

Internal gases, CO_2 and O_2, from 10 fruit samples per treatment were evaluated by withdrawing a 10-mL internal fruit gas sample from the fruit columella using a syringe while the fruit was submerged in room temperature water. These samples were analyzed using a gas chromatograph (Hewlett Packard 5890A, Agilent Technologies) fitted with a CTR column (Cole-Parmer, Vernon Hills, IL) and a thermal conductivity detector. The gas flow rates for helium and air were 80 and 350 $mL \cdot m^{-1}$, respectively. Temperatures of the oven, injector, and detector were 70, 250, and 250 °C, respectively. Weight loss of 10 fruit per treatment was measured during storage, and results were expressed as a percentage of initial weight.

Gloss measurements. A reflectometer (micro-TRI-gloss; BYK-Gardner, Colombia, MD) was used to evaluate gloss units, which are an indication of visual shine for coating formulations on test paper (140 × 254 mm) and on the fruit. For test papers, an aliquot of 0.5 mL of each coating was spread on a 0.05-mm-thick polished paper (Leneta Co., Mahwah, NJ) with a 0.1-mm castor (BYK-Gardner) with a speed of 1 $cm \cdot s^{-1}$. The control consisted of uncoated paper. Five papers per treatment were used, and measurements were repeated five times per paper at different positions. The reflectance was measured at angles of 20° for test papers (Bai et al., 2003a) and 60° for fruit (Bai et al., 2003b) according to the manufacturer's instructions because the paper is flat and the fruit is round. For coated fruit, a case with a circular 19-mm-diameter orifice was attached to the equipment to accommodate the round fruit shape. For gloss measurements, 10 fruit per treatment were used and measurements were performed twice at opposite points of the equatorial region of the fruit.

Sensory visual shine analyses. Coated fruit were visually evaluated for shine by 25 and 19 panelists for 'Nova' and 'Unique' fruit, respectively, for comparison with the gloss units measured with the reflectometer. Panelists consisted of laboratory staff familiar with the assessment of citrus products. For 'Nova', two groups of four fruit were presented, with each group containing all four treatments, and panelists were asked to pair fruit by appearance (tetrad test) (Ennis, 2012). Then, panelists were asked to rank the pairs for shine in decreasing order of shine intensity (most shine to least shine). For 'Unique'

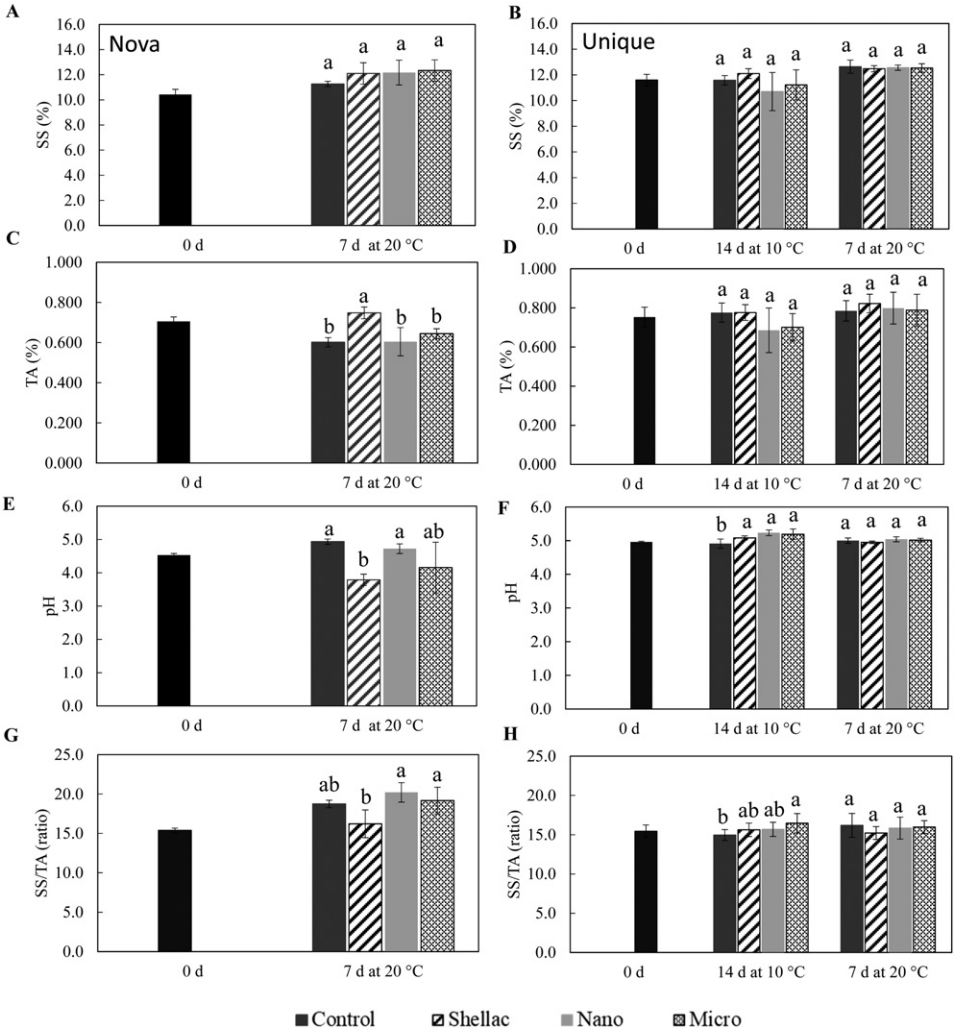

Fig. 2. Nanoemulsions and microemulsions of carnauba wax compared with a commercial shellac microemulsion to determine effects on citrus fruit sugar and acid measurements. (**A**, **B**) Soluble solids (SS), (**C**, **D**) titratable acidity (TA), (**E**, **F**) pH, and (**G**, **H**) SS/TA ratio values for 'Nova' mandarin and Unique' tangors, respectively, that were uncoated and coated with different coatings (five composite replicates of three fruit each). Columns with different letters are significantly different within each storage period according to Duncan's test ($P < 0.05$).

tangors, panelists were also instructed to squeeze fruit between the middle finger and thumb to rate firmness/hardness of the fruit using a 10-point scale (0 soft to 10 hard). During both tests, each fruit was presented with a three-digit randomized code on a tray under daylight illumination.

Sensory flavor evaluation. 'Unique' fruit were juiced as described previously and frozen at 20 °C. On the day of the panel, the juice was thawed overnight at 5 °C, and 40 mL of juice was poured in 120-mL cups with lids (Solo, Urbana, IL). Coded juice samples were presented to panelists at 14 ± 1 °C, following the William's design. An additional cup of control juice was presented and labeled as "warm up." Panelists were instructed to first taste the "warm up" sample to familiarize their taste buds with the juice (O'Mahony et al., 1988). Then, they were instructed to take a sip from each coded cup and rank the samples for overall flavor from best to worst. They were allowed to taste as many times as necessary. Finally, a question was asked about any off-flavor perceived in each sample. Panelists were asked to rate off-flavor from 1 (no off-flavor) to 10 (extreme off-flavor) and to describe the off-flavor if perceived. Tasting was performed in isolated booths under red lighting. Data were recorded using Compusense Cloud (Compusense, Guelph, ON, Canada). Twenty-four panelists participated in a flavor evaluation of 'Unique' juice.

Statistical analyses. Physical and chemical parameters were analyzed using a univariate parametric analysis of variance (ANOVA) at each storage time, a multiple comparison Duncan test or Games-Howell (according to homogeneity of variance assumed or not), and a *t* test for paired samples. The significance level for all analyzes was 5%. IBM SPSS Statistics (subscription trial free version; IBM Corp., Armonk, NY) and R (R Core Team, Vienna, Austria) software were used. Ranked sensory data were analyzed using a critical absolute rank sum differences table at $P < 0.05$ (Newell and MacFarlane, 1987). Tetrad test (Ennis, 2012) data were analyzed using an approximation equation for tetrad (Z-test) at $P = 0.05$. A comparison of sensory firmness scores was performed using a nonparametric ANOVA and multiple comparison of Kruskal-Wallis because of the ordinal level of the variables and four independent samples in the experiment ($P < 0.05$). Off-flavor ratings were analyzed by the ANOVA in a mixed model with random panelists using a statistical package (Senpaq version 5.01; Qi Statistics, Reading, UK). Principal component analysis (PCA) was performed using a multivariate statistical package (JMP version 13; SAS Institute, Cary, NC) to test the separation among coating treatments and storage times based on the volatile compounds.

Results and Discussion

Weight loss. A major function of coatings on fruit is to retard water loss, which is measured as weight loss. Microemulsion (conventional) and nanoemulsion (experimental) carnauba wax coatings resulted in less weight loss compared with control and shellac-coated fruit, which were not different from each other, during the first experiment with 'Nova' mandarins (Fig. 1A). The microemulsion fared slightly better than the nanoemulsion for retarding weight (water) loss; therefore, perhaps, the more numerous and smaller lipid micelles were more permeable to water vapor than the larger lipid micelles of the microemulsion. For the second experiment with 'Unique' tangors, fruit weight loss was minimal after 14 d at 10 °C (less than 2%) (Fig. 1B), with the control showing the most weight loss, but it was not different from that of the

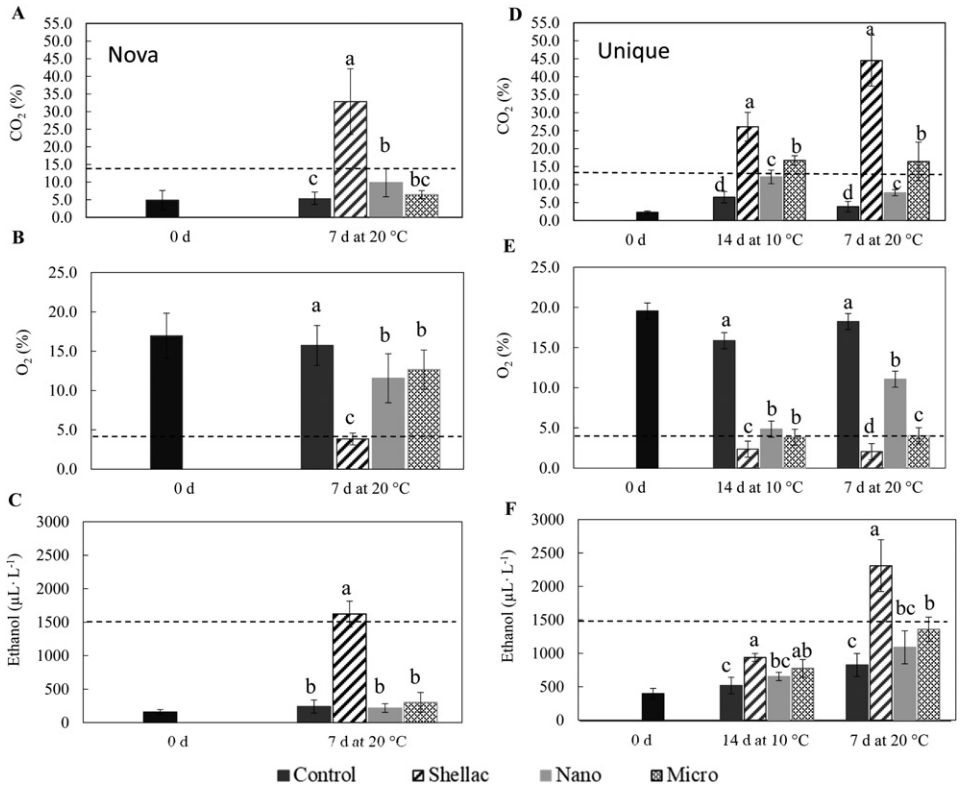

Fig. 3. Nanoemulsions and microemulsions of carnauba wax compared with a commercial shellac microemulsion to determine citrus fruit internal atmosphere modifications in (**A**, **D**) internal CO_2 for 'Nova' mandarins and 'Unique' tangors, respectively, (**B**, **E**) internal O_2 (10 fruit/treatment) for 'Nova' mandarins and 'Unique' tangors, respectively, and (**C**, **F**) juice ethanol values for 'Nova' mandarins and Unique' tangors, respectively (five replicates of three fruit each). Columns with different letters are significantly different for each storage period according to Duncan's test or the Games Howell test ($P < 0.05$). Dashed line represents the critical level according to Hagenmaier (2002) at which the mandarin flavor may be affected (internal CO_2 and juice ethanol >14% and >1500 $\mu L \cdot L^{-1}$, respectively, and O_2 <4%).

shellac. At the end of storage (14 d at 10 °C + 7 d at 20 °C), the microemulsion followed by the nanoemulsion coating exhibited less weight loss when compared with uncoated fruit, with the shellac coating being intermediate between control and the waxes.

Carnauba wax nanoemulsions and microemulsions are less permeable to water vapor than shellac due to its lipophilic nature. The solid lipid structure is dense and restricts water diffusion (Morillon et al., 2002); therefore, the results are not surprising. Several studies have reported that carnauba-based coatings decrease water loss (Jo et al., 2014; Kim et al., 2014).

Sugars and acids. Sweet and sour tastes, attributed to sugars and acids, are important to fruit flavor quality. There were minor statistical, but no practical, differences in the SS, TA, pH, and ratio among treatments (Fig. 2). For 'Nova' mandarin coated fruit (Fig. 2A, C, E, and G), there were no differences in the SS between coatings (Fig. 2A); however, shellac had the highest TA and lowest pH (not different from the microemulsion), which resulted in the lowest SS/TA (not different from control) (Fig. 2C, E, and G). Generally, although not tested statistically, there was a slight increase in the SS, pH, and SS/TA and a decrease in the TA (except shellac for TA and pH and microemulsion for pH) after 7 d at 20 °C. Similar results were reported by Obenland et al. (2011). The authors associated the higher SS/TA with the slightly superior flavor for mandarin fruit stored at 8 °C for up to 6 weeks and 1 week at 20 °C.

For 'Unique' tangors, there were also no substantial differences in the SS, TA, pH, or ratio among treatments (Fig. 2B, D, F, and H). However, the pH and SS/TA ratio for uncoated fruit were statistically the lowest among the treatments after 14 d at 10 °C (not different from shellac or nanoemulsion for SS/TA) (Fig. 2F and H, respectively), and there were no differences after an additional 7 d at 20 °C. Similarly, no significant differences in SS or TA were detected for different storage temperatures or during the storage period for 'Valencia' oranges coated with shellac, a cellulose-based coating, or uncoated fruit (Baldwin et al., 1995b).

Internal gases. Fruit coatings can result in a modified fruit internal atmosphere that can affect flavor. The volatile ethanol is discussed here because it is affected by O_2 levels and is an indicator of anaerobic respiration and the resulting off-flavor. For fruit in these two experiments, internal CO_2 and juice ethanol generally increased and internal O_2 decreased during storage (Fig. 3). For 'Nova' mandarins, the highest levels of CO_2 and ethanol and the lowest O_2 level were found with the shellac treatment, suggesting anaerobic respiration. The lowest levels of CO_2 and ethanol and the highest O_2 level were found in uncoated control fruit. The nano- and microemulsion carnauba wax coatings were intermediate and not different from each other (Fig. 3A–C). High ethanol has been directly linked to off-flavor and altered flavor (Baldwin et al., 1995b; Hagenmaier, 2000, 2002; Hagenmaier and Goodner, 2002; Ke and Kader, 1990). Hagenmaier (2002) coated several mandarin hybrids (eight cultivars) with wax (polyethylene and candelilla) and resin (shellac) and reported that a sensory taste panel found that fruit coated with the low gas permeability coating (shellac) had less fresh flavor compared with those coated with higher gas permeability coatings (polyethylene and candelilla waxes). The author demonstrated that the mandarin flavor may be affected when the internal CO_2 is higher than 14%, the internal O_2 is lower than 4%, and the juice ethanol content is higher than 1500 $\mu L \cdot L^{-1}$ after 7 d storage at 20 °C (see dashed lines in Fig. 3A–F).

'Unique' tangors showed the same trend as 'Nova' mandarins: fruit coated with shellac showed the lowest O_2 along with highest CO_2 and ethanol levels, followed by the carnauba microemulsion and nanoemulsion (Fig. 3D–F), especially after 7 d at 20 °C. Uncoated fruit had the lowest ethanol and CO_2 levels, along with the highest O_2 level. There were no differences between the nanoemulsion and control for ethanol levels or between the nanoemulsion and the microemulsion for O_2 or ethanol after 14 d of cold storage and after the simulated marketing conditions for ethanol. Lower ethanol levels in the

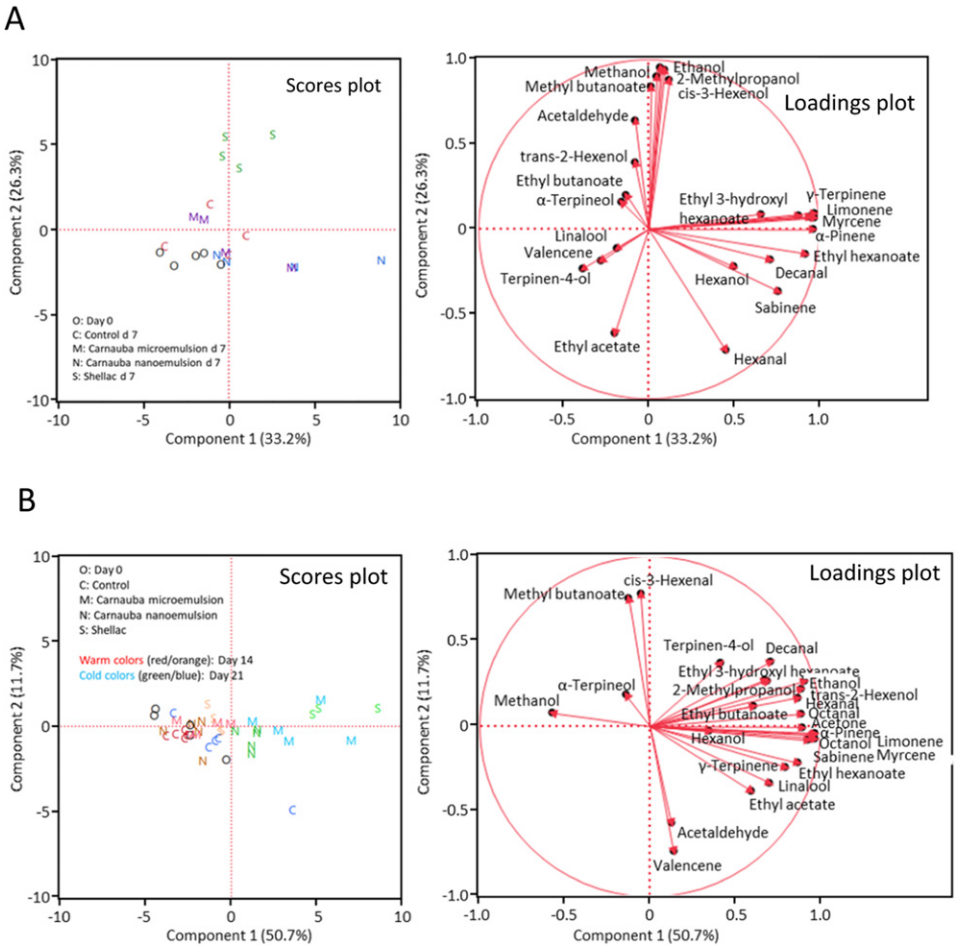

Fig. 4. Effects of shellac and carnauba coatings on citrus fruit aroma volatiles. Principle component analysis of 26 aroma volatile compounds measured in the juice of 15 citrus fruit (five composite replicates of three fruit each) for untreated fruit [control (C)] or fruit coated with three different coatings and stored at 20 °C [day 0 (O)]. (**A**) 'Nova' mandarins stored for 7 d at 20 °C [C, shellac (S), nanoemulsion (N), and microemulsion (M)] and (**B**) 'Unique' tangors stored for 14 d at 10 °C (red/orange C, S, N, and M) or for 14 d at 10 °C plus 7 d at 20 °C (green/blue C, S, N, and M).

nanoemulsion coating could indicate better flavor, similar to that of uncoated fruit. Therefore, both carnauba coatings in this study were shown to be suitable for mandarins and tangors. Similar results were reported by Navarro et al. (2007) for 'Valencia' oranges and 'Marisol' tangerines. These authors reported that internal CO_2 and ethanol were highest for fruit coated with shellac.

Shellac coatings can reduce gas exchange, thus modifying the internal atmosphere and creating an anaerobic/fermentative environment (Baldwin et al., 1995a; Hagenmaier, 2000; Hagenmaier and Baker, 1994). Usually resin coatings have low oxygen permeability properties, and shellac and wood resin are generally less permeable than waxes such as polyethylene, candelilla (Hagenmaier, 2002), and carnauba (Assis et al., 2008; Lin and Zhao, 2007). Therefore, lipid-based coatings, such as carnauba wax, present a more effective moisture barrier and are relatively permeable to gases (Assis et al., 2008; Lin and Zhao, 2007), resulting in less off-flavor.

Low oxygen atmospheres have been shown to result in an increased production of ethanol, methanol, and acetaldehyde in citrus fruit (Baldwin et al., 1995b; Davis, 1970; Shaw et al., 1990). To summarize this study, carnauba wax emulsions exhibited intermediate modifications of the fruit internal atmosphere of uncoated controls and shellac, presented a better moisture barrier than shellac, and resulted in less ethanol, with the nanoemulsion ethanol levels not different from those of uncoated fruit (Fig. 3C and F).

Aroma volatile analysis. The PCA plots of aroma volatiles of 'Nova' mandarins and 'Unique' tangors are shown in Fig. 4A and B, respectively. The PCA for 'Nova' mandarins (Fig. 4A) explained 59.5% of the data variation (33.2% and 26.3% for components 1 and 2, respectively) and 62.4% of the variation (mostly in component 1) for 'Unique' tangors (Fig. 4B), offering more evidence of the altered internal atmosphere due to coatings. For 'Nova' mandarins, shellac coatings were associated with ethanol, methanol, and acetaldehyde, indicating fermentation and other volatiles. The carnauba wax emulsions were clustered with control and 0-d samples or further down and to the right on component 1, especially one nanoemulsion outlier, that was associated with common citrus terpenes, esters, and an aldehyde (limonene, myrcene, α-pinene, γ-terpinene, ethyl hexanoate, and decanal among others) (Fig. 4A). For 'Unique' tangors, there were not many volatile differences after 14 d at 10 °C for any of the treatments, which were similar to those of 0-d juice, because the metabolism at this temperature is relatively slow (Fig. 4B). However, after cold storage followed by the simulated marketing period at 20 °C, there were more differences between treatments. The aroma volatiles in the juice from 0-d fruit, controls, and the carnauba nanoemulsion treatment were associated with fewer volatiles (on the left side of component 1) but associated with methanol and α-terpineol. The juice from fruit coated with the microemulsion and that from fruit coated with shellac were associated with more aroma volatiles on the right side of component 1, including acetaldehyde, ethylacetate, ethanol, and acetone (Fig. 4), which are associated with anaerobic respiration. One control outlier was also associated with acetaldehyde as well as valencene. Although the other volatiles shown in Fig. 4 are mostly desirable and comprise the citrus aroma, the coatings may have trapped them and/or affected their synthesis, resulting in an altered flavor profile (as evidenced for 0-d and control uncoated fruit juice) and possible off-flavor.

Fruit flavor evaluation. During the second experiment with 'Unique' tangors, the juice used to measure the aroma volatiles was also evaluated by a sensory panel for flavor (Fig. 5A–C). After 14 d of cold storage, control fruit juice was ranked

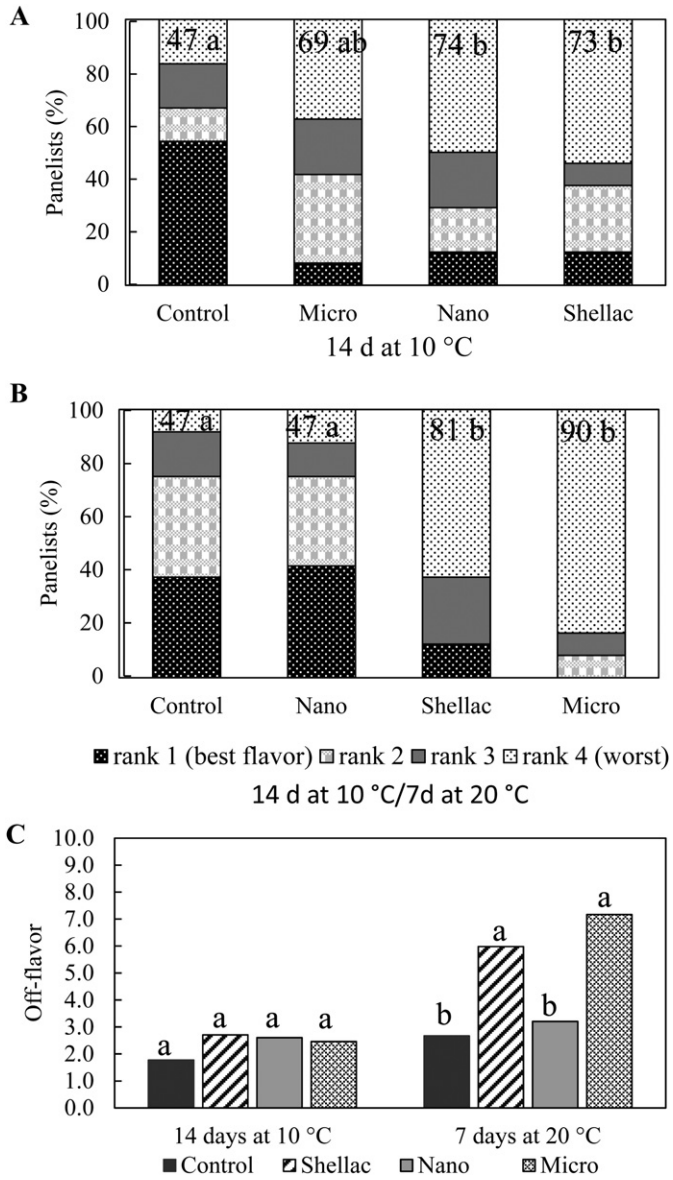

Fig. 5. Nanoemulsions and microemulsions of carnauba wax compared with a commercial shellac microemulsion for effects on citrus fruit juice flavor. Fruit juice was ranked for the tangerine flavor of 'Unique' tangors after 14 d at 10 °C (**A**) and after an additional 7 d at 20 °C (**B**). Columns with different letters are significantly different according to the critical absolute rank sum differences table (value on figure bars, shown in order of ranking) at $P < 0.05$ (data shown in order of ranking) (Newell and MacFarlane, 1987) for the four samples as determined by 24 panelists. Color shading depicts the percentage of panelists' selections. (**C**) Juice rated for off-flavor using a scale of 1 to 10, where 1 = none and 10 = extreme. Numbers (mean rating, n = 24) followed with the same letter are not statistically different according to Fisher's least significant difference test ($P < 0.05$).

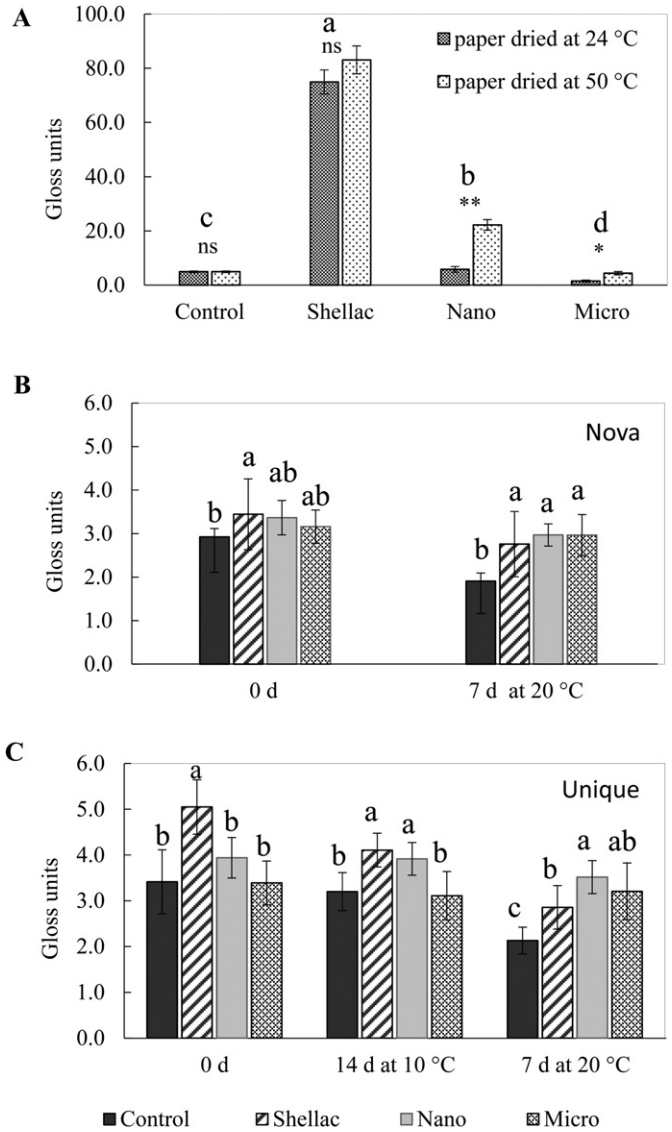

Fig. 6. Nanoemulsions and microemulsions of carnauba wax that impart shine measured as gloss units by a gloss meter. (**A**) Gloss units for uncoated (control) and coated black paper (five test papers with five measurements each). Differences were determined by the *t* test (*$P < 0.05$, **$P < 0.1$). (**B**) Gloss units for uncoated (control) and coated 'Nova' mandarins (**C**) and for 'Unique' tangors (10 fruit/treatment with two measurements/fruit). Coatings: shellac (shellac microemulsion), nano (carnauba nanoemulsion), and micro (carnauba microemulsion). For each storage period, columns with different letters are significantly different according to Duncan's test or the Games Howell test at $P < 0.05$.

as having the best mandarin flavor (lowest value), although it was not different from that of the carnauba microemulsion; however, the carnauba nanoemulsion and shellac-coated fruit were ranked as having the worst mandarin flavor (highest value), although it was not different from that of the carnauba microemulsion (Fig. 5A). For off-flavor at 14 d, there were no differences between treatments (Fig. 5C). This reflects the volatile data that showed few differences between treatments after cold storage (Fig. 4). After 14 d of cold storage followed by 7 d at 20 °C, control fruit juice and the carnauba nanoemulsion-coated fruit juice were ranked highest for mandarin flavor, and the shellac-coated and microemulsion-coated fruit juices were ranked the lowest (Fig. 5B). This also reflects the increased volatile changes after the simulated marketing period at 20 °C (Fig. 4). Similarly, regarding off-flavor, the control and carnauba nanoemulsion-treated fruit juices were rated lowest for off-flavor, and the shellac-treated and microemulsion-treated fruit juices were rated highest (Fig. 5C). This is consistent with the ethanol and other volatile levels, especially for shellac. The CO_2, O_2, and ethanol levels for the carnauba microemulsion were at or near levels that would predict flavor

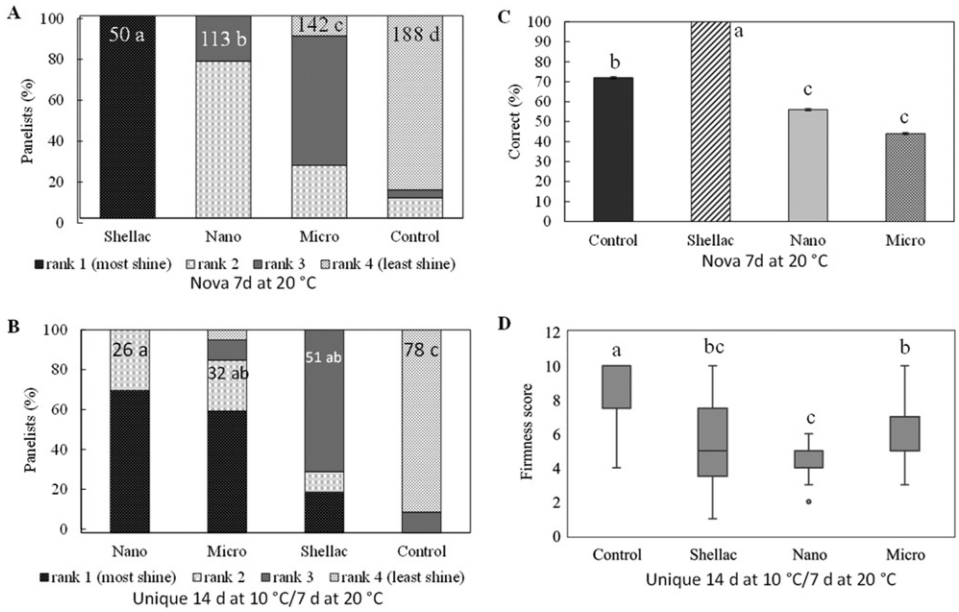

Fig. 7. Nanoemulsions and microemulsions of carnauba wax that impart visual shine to citrus fruit. Sensory shine ranking for 'Nova' mandarins stored for 7 d at 20 °C (**A**) and 'Unique' tangors stored for 10 d at 10 °C and then for 7 d at 20 °C (**B**) at end of the storage period. Columns with different letters were significantly different according to the critical absolute rank sum differences table at $P < 0.05$ (value on figure bars, shown in order of ranking) (Newell and MacFarlane, 1987). Color shading depicts the percentage of panelists' selections. (**C**) Sensory tetrad test for 'Nova' mandarins at the end of the storage period. Columns with different letters were significantly different according to the approximation equation for tetrad (Z-test) at $P \leq 0.05$. (**D**) Sensory firmness perception for 'Unique' tangerine using a 10-point scale (0 soft to 10 hard) at the end of the storage period. Columns with different letters are different according to the Kruskal-Wallis multiple comparisons test ($P < 0.05$).

problems, but it is surprising that there were no flavor differences compared to shellac-coated fruit, which induced a much more extreme modification of the atmosphere than did the carnauba microemulsion, resulting in higher ethanol. Off-flavor descriptors for the shellac-coated and microemulsion-coated fruit were "rancid, "fermented," "chemical," "rotten fruit," and "soapy," which might be explained by higher amounts of ethyl acetate, ethyl hexanoate, ethyl-3-hydroxy hexanoate, and some aldehydes (Plotto et al., 2008).

Gloss analyses. Fruit visual shine boosts sales; therefore, coatings that impart shine are sought after by the coating industry, especially for citrus. During preliminary tests with paper sheets, gloss units on coated paper sheets increased when hot air was used to dry the coating surface and were generally higher when coatings were dried at 50 °C compared to 20 °C (Fig. 6A), which is significant for the two carnauba emulsions. This indicates that the application of heated air helps coatings to dry and tends to impart more visual shine to the fruit. In fact, heated air-drying tunnels are often used for citrus by the industry (Hall, 2012). Shellac, as expected, had the highest gloss, which was significantly different from that of carnauba wax nanoemulsions and microemulsions and the uncoated paper (Fig. 6A).

For gloss on 'Nova' mandarin fruit (Fig. 6B), the shellac coating showed higher gloss units than the uncoated control initially; the gloss units of the carnauba wax coatings were not different from those of the shellac coatings or control. However, after 7 d of storage, all coatings showed more gloss units than the control, and there were no differences among coating treatments. For 'Unique' tangors (Fig. 6C), initially, the shellac showed the highest gloss units, which were significantly different from those of the other treatments, which were not different from each other. After 14 d of storage at 10 °C, shellac and the carnauba wax nanoemulsions showed the highest gloss units, with no difference between them, and there was no difference between the gloss units of the carnauba microemulsion and control. However, after 7 d at 20 °C, the nanoemulsion showed the highest gloss units; the gloss units of the microemulsion were not different from those of the nanoemulsion or shellac. Citrus fruit loss of gloss units was observed for all treatments over time in storage; however, this loss was greater for shellac-coated fruit.

Similar results were reported by Navarro et al. (2007) for 'Valencia' sweet oranges and 'Marisol' tangerines treated with different coatings; the fruit gloss was highest with the shellac coating. In another study, after storage, the shellac gloss/shine decreased but was still shinier than that of the uncoated fruit. For that study, the shellac coating provided fruit with more shine and exhibited better weight loss control when compared with commercial cellulose-based coatings (Baldwin et al., 1995b).

Sensory visual shine analyses. For 'Nova' mandarins, shellac-coated fruit were ranked highest for visual shine by panelists after 7 d at 20 °C, followed by carnauba nanoemulsion, carnauba microemulsion, and the uncoated control (Fig. 7A). For 'Unique' tangors, the carnauba nanoemulsion had the highest visual shine score, although it was not different from that of the microemulsion; however, its shine score was higher than that of the shellac, and all shine scores were higher than that of the uncoated fruit (Fig. 7B) after 14 d at 10 °C and 7 d at 20 °C. There was no difference in the visual shine with the carnauba microemulsion and shellac. During the sensory pairing test (tetrad) with 'Nova' mandarins, the panelists recognized shellac-coated and uncoated fruit but could not differentiate between carnauba wax microemulsions and nanoemulsions based on the appearance of coated fruit (Fig. 7C).

The results for ranking based on visual shine are in agreement with those of Navarro et al. (2007), who reported that shellac-coated fruit ranked third for appearance (out of five treatments) and was considered too shiny. At the end of the cold storage (14 d at 10 °C) and simulated marketing conditions (7 d at 20 °C), uncoated tangors were more firm (hard, dried out). Those coated with the microemulsion and the nanoemulsion were the softest. Those coated with shellac were not different from those coated with either of the carnauba emulsions (Fig. 5D).

In conclusion, carnauba wax emulsion coatings allowed more gas exchange, resulting in fewer fruit internal atmosphere modifications, more sustainable shine (as measured by gloss

units), and reduced water loss more effectively than a commercial shellac microemulsion on citrus fruit. Carnauba coatings did not affect the fruit sugar and acid levels, whereas shellac effects were minimal. The shellac coating induced the lowest O_2 and highest CO_2 and ethanol levels in fruit, which indicated anaerobic respiration and could predict undesirable flavor changes, as confirmed by a sensory panel. The panel found that the uncoated controls and the nanoemulsion-coated fruit had the most tangerine flavor and least off-flavor. An aroma volatile analysis showed that the shellac and microemulsion resulted in increased volatile levels, indicating alterations of the normal volatile profile. When the two carnauba coatings were compared, the microemulsion showed slightly better water loss control and the nanoemulsion generally exhibited higher gloss/shine, fewer modifications of the fruit internal atmosphere, fewer alterations of the aroma volatile profile, and lower ethanol levels. This resulted in better flavor quality for nanoemulsion-coated fruit juice, which was not different from the uncoated control fruit juice, which represented unaltered flavor. The effect on flavor is important because the use of coatings often detrimentally affects flavor, which affects repeat sales (Baldwin et al., 2014). For the carnauba emulsions, the materials used to make both emulsions are very similar, but the equipment is different. The nanoemulsion needs a closed reactor and a high-pressure homogenizer, which is a financial investment for coating companies, but the cost of the material is the same. The preparation time is ≈3 h for the nanoemulsion but ≈30 m for the microemulsion. The use of edible coatings is an environmentally friendly technology that can reduce postharvest losses and maintain fruit quality when used appropriately, and the carnauba nanoemulsion is as good as or better than the carnauba microemulsion for shine and flavor. Future work should evaluate decay control for carnauba and shellac microemulsions compared with the carnauba nanoemulsion for citrus fruit with and without antimicrobial essential oils.

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Edible Coatings as Carriers of Antibrowning Compounds to Maintain Appealing Appearance of Fresh-cut Mango

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ADDITIONAL INDEX WORDS. *Aloe vera*, carboxymethylcellulose, browning, sensory evaluation, shelf life, whey protein isolate

SUMMARY. Fresh-cut mango (*Mangifera indica*) slices and chunks garner an exotic image and are highly appreciated for their unique flavor and nutritional value. However, processors tend to use firm unripe mangoes to achieve shelf life of 10 to 14 days, which compromises eating quality. The post-processing life of ripe fresh-cut mangoes is limited by tissue softening, translucency, and browning. The current study was undertaken to investigate whether edible coatings can extend the shelf life of fresh-cut mangoes processed at an eating-ripe stage. Three edible coatings, carboxymethylcellulose (1% w/v), aloe (*Aloe vera*) powder (2% w/v), and whey protein isolate (2% w/v), supplemented with calcium ascorbate 2% w/v (firming agent) and the antioxidants citric acid (0.8% w/v) and acetyl-*N*-cysteine (0.4% w/v), were used. The mixture of antibrowning agents, whether applied alone or with the edible coatings, was the most effective at reducing slice browning up to 10 and 11 days at 5 °C for 'Tommy Atkins' and 'Kent', respectively. In general, there were no differences in firmness and flavor among the three edible coatings. Calcium ascorbate alone did not suppress browning consistently, whereas citric acid appeared to be the ingredient having the greatest antibrowning effect on slice quality. Citric acid can easily be used by processors of fresh-cut mangoes to prevent browning.

Mangoes (*Mangifera indica*) represent a small part of the fresh-cut industry but their popularity is growing, as reflected by the increase in sales by 8% between 2013 and 2017 in the United States (National Mango Board, 2017), and recently ranking seventh among fresh-cut fruit sales in 2019 (Van Den Broek, 2020). Their appealing flavor and texture, and the added convenience that ready-to-eat fresh-cut products offer compared with whole fruit, are some of the main contributing drivers (Brecht et al., 2017).

The process of wounding whole fruit tissue to obtain fresh-cut fruit stimulates the development of undesirable color changes, due to enzymatic browning, and tissue softening. It also provides a substrate for microbial growth, increasing the risk of developing pathogens (Baldwin, 2007). Therefore, fresh-cut fruit shelf life is considerably shorter than for whole fruit. To delay fresh-cut fruit deterioration, processors tend to cut mangoes on reception without preripening to avoid overripe, soft, bruised, and decayed fruit (Brecht et al., 2017). Furthermore, fresh-cut mangoes must be maintained at no higher than 5 °C, both to prolong shelf life and to reduce the risk associated with potential proliferation of microbial food-borne human pathogens (U.S. Food and Drug Administration, 2017). Under these conditions, unripe fresh-cut mangoes do not fully develop their desirable sensory attributes, which may negatively affect repeat purchase by consumers. In this context, the fresh-cut mango industry needs to reach a balance between acceptable shelf life and appealing organoleptic features, to offer a high-quality product that meets the expectations of all stakeholders involved. Processing riper fruit might be a way to achieve this goal, but at the same time can increase susceptibility to wounding during processing and shorten shelf life (Beaulieu and Lea, 2003; Brecht et al., 2017; Ngamchuachit et al., 2015).

The use of edible coatings (ECs) for the purpose of extending shelf life and improving quality of fresh-cut fruit is an extensively reviewed strategy (Baldwin and Brecht, 2020; Dea et al., 2012; Ghidelli and Pérez-Gago, 2018; Yousuf et al., 2018). Delay of flesh browning and tissue softening are some of their most notable benefits. Antibrowning and/or firming agents such as calcium ascorbate, citric acid, and *N*-acetyl-L-cysteine are

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
1	%	g/100 mL	1
29,574	fl oz	µL	3.3814×10^{-5}
29.5735	fl oz	mL	0.0338
3.7854	gal	L	0.2642
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
0.4536	lb	kg	2.2046
4.4482	lbf	N	0.2248
28.3495	oz	g	0.0353
1	ppm	$mg \cdot L^{-1}$	1
1	ppm	$\mu L \cdot L^{-1}$	1
(°F – 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

frequently used in the fresh-cut industry, and their incorporation into ECs is known as an effective tool to improve their efficacy. Polysaccharide-based ECs have been widely reported as good matrices to incorporate compounds with antibrowning properties in fresh-cut mango and other fruit (Benítez et al., 2015; Plotto et al., 2010; Robles-Sánchez et al., 2013; Saba and Sogvar, 2016), whereas the application of coatings based on proteins is more limited, and only a few studies with apple (*Malus domestica*) and persimmon (*Diospyros kaki*) fruit can be found in the literature (Alves et al., 2017; Ghidelli et al., 2010; Pérez-Gago et al., 2006).

This study aimed at determining how the shelf life of fresh-cut mangos processed at an eating-ripe stage could be extended by treatments with antioxidants and/or firming compounds incorporated into ECs to offset the expected shelf life limitations caused by using riper fruit. For that purpose, the influence of ECs on physicochemical and sensory properties of fresh-cut mango were analyzed during storage at 5 °C for 14 d.

Materials and methods

Preparation of the antibrowning coating solutions. All solutions were prepared with sterile deionized water and all ingredients were food grade (Table 1). Calcium ascorbate [CaAS (Sigma-Aldrich, St. Louis, MO)] was diluted to 2% (w/v) for 20 min at room temperature. The antioxidant solution (ANTIOX), was a mixture consisting of CaAS (2%), citric acid [0.8% w/v (Sigma-Aldrich)], and *N*-acetyl-L-cysteine [0.4% w/v (Sigma-Aldrich)] dispersed at room temperature for 20 min. The ANTIOX solution was applied alone or in combination with ECs. Carboxymethylcellulose sodium salt [CMC (Sigma-Aldrich)], was dissolved (1% w/v) in warm water for 2 h, and after cooling, dextrin [0.5%, w/v, dextrin Type II from corn (*Zea mays*) (Sigma-Aldrich)] was added and the mixture was stirred for an additional 5 min. Aloe powder [ALOE (S.W. Basics, Brooklyn, NY)] was dissolved at 2% (w/v) for 20 min. Whey protein isolate [WPI (The Isopure Co., Downers Grove, IL)] was dissolved (2% w/v) for 30 min at 90 °C to induce protein denaturation. On cooling, glycerol [0.5% w/v (bioWORLD, Dublin, OH)] was added and the solution was homogenized for 4 min at 12,000 rpm using a homogenizer (Bio-Gen PRO200; PRO Scientific, Oxford, CT). ANTIOX solution was added to each EC solution at room temperature by stirring for 10 min. The concentrations of the different solutions were selected based on previous studies (Blanco-Díaz et al., 2014; Plotto et al., 2010). The pH of each solution was measured in duplicate (Table 1).

Fruit source and processing. 'Tommy Atkins' and 'Kent' mangoes from Mexico were purchased from the importer on arrival (Coast Tropical, Homestead, FL) and brought to the U.S. Horticultural Research Laboratory (Fort Pierce, FL). Fruit had been subjected to quarantine hot water treatments mandated by the U.S. Department of Agriculture (2016), and maintained at 12 °C during shipping. On reception, a subsample of mangoes was randomly selected and kept at room temperature to assess ripeness level by nondestructive compression firmness measurement using a texture analyzer (TA.XT *plus*; Stable Micro Systems, Godalming, UK) equipped with a 50-kg load cell and with a 5-cm-diameter flat plate (TA-25, Stable Micro Systems), and calibrated with a 5-kg weight. The tests were performed at a crosshead speed of 0.8 $mm \cdot s^{-1}$ and force was recorded at 2.5 mm deformation (Dea et al., 2013). Depending on subsample initial firmness, fruit were stored either at 13 °C (if firmness was 30 to 40 N for 'Kent') or 20 °C (if firmness was > 40 N for 'Tommy Atkins'). Whole fruit compression firmness was monitored and when at least 250 mangoes ranged between 30 and 50 N (average 43.64 ± 0.40 N for 'Tommy Atkins', and 38.25 ± 0.58 N for 'Kent'), processing was carried out. An average firmness of 35 N before processing was previously determined by Dea et al. (2013), and then confirmed in preliminary studies (unpublished data) as an appropriate ripeness stage for fresh-cut mango in terms of handling, visual quality, and quality maintenance during storage. In addition, simulating industry practice, whole fruit firmness was also measured after removal of the peel by penetration using an 11-mm probe. Any fruit softer than 5.5 N for 'Tommy Atkins' and 7 N for 'Kent', and firmer than 30 N (both cultivars) were discarded. Average penetration firmness was 14.12 ± 0.38 N for 'Tommy Atkins', and 14.96 ± 0.47 N for 'Kent'. Mangoes were prewashed with a fruit cleaner (JBT 395; JBT FoodTech, Lakeland, FL), then sanitized with 100 $\mu L \cdot L^{-1}$ peroxyacetic acid [PAA (Jet-Oxide; Jet Harvest Solutions, Longwood, FL)] in warm water (30 ± 3 °C) for 3 min and air dried. Fruit were stored at 5 °C overnight. Processing was carried out at 5 °C in a cold room and all surfaces and tools were sanitized with 300 $mg \cdot L^{-1}$ sodium hypochlorite (NaOCl) acidified with 2 M citric acid solution (pH 6.5), changing the solution every 10 fruit. Mangoes were manually peeled to a depth of ≈2 mm to ensure complete removal of the subepidermal

Table 1. Composition and pH of the different coating solutions applied to fresh-cut mango slices.

Solution[z]	Edible coating and concn (%)	Antibrowning agent and concn (%)	pH
CaAS	—	Calcium ascorbate, 2%	6.52
ANTIOX	—	Calcium ascorbate, 2% Citric acid, 0.8% *N*-acetyl-L-cysteine, 0.4%	3.56
CMC	Carboxymethylcellulose, 1% + maltodextrin, 0.5%	ANTIOX	3.75
ALOE	Aloe powder, 2%	ANTIOX	3.59
WPI	Whey protein isolate, 2% + glycerol, 0.5%	ANTIOX	3.71

[z]CaAS = calcium ascorbate; ANTIOX = calcium ascorbate + citric acid + *N*-acetyl-L-cysteine; CMC = carboxymethylcellulose + ANTIOX; ALOE = aloe + ANTIOX; WPI = whey protein isolate +ANTIOX.

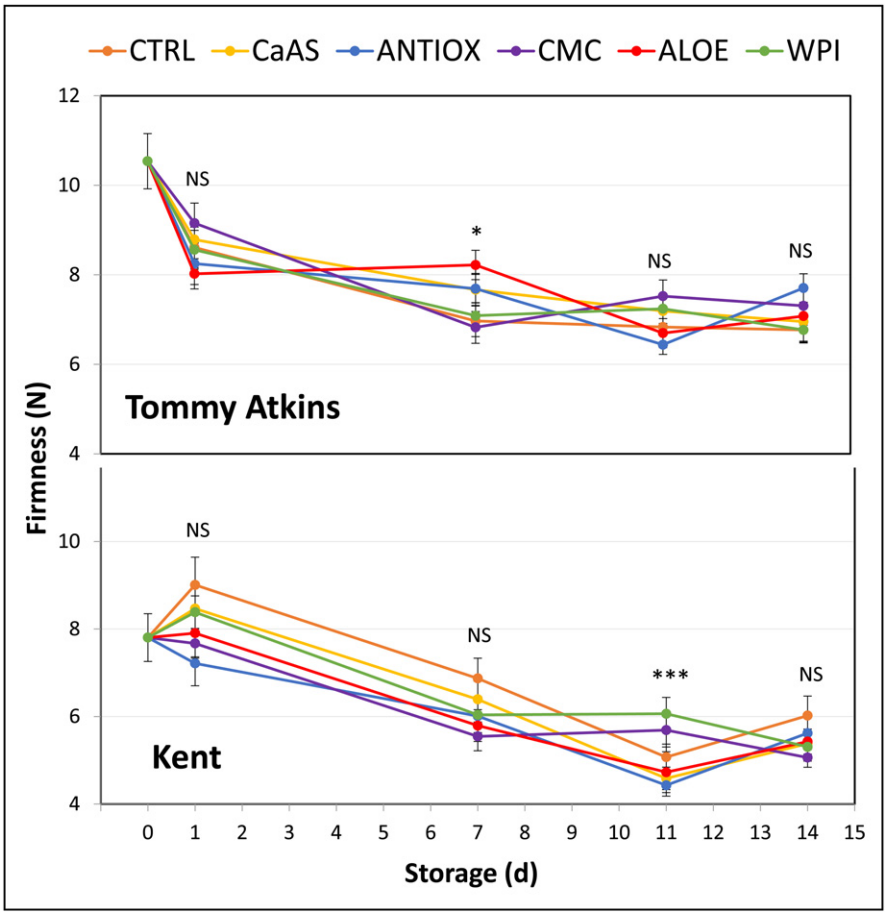

Fig. 1. Firmness of fresh-cut 'Tommy Atkins' and 'Kent' mango slices coated with different solutions stored at 5 °C (41.0 °F) for 14 d (CTRL = control; CaAS = calcium ascorbate; ANTIOX = antibrowning mix; CMC = carboxymethylcellulose + ANTIOX; ALOE = aloe + ANTIOX + ANTIOX; WPI = whey protein isolate + ANTIOX). Values are means (with SE) of 10 measurements per four containers; NS = not significant between coating treatments and within a storage time; *, **, ***significant at $P < 0.05$, 0.01, or 0.001, respectively; 1 N = 0.2248 lbf.

tissue, which tends to discolor (brown) during storage. Each fruit was cut into halves on each side of the seed and then into uniform slices of 5/8-inch thickness using a commercial electric meat slicer (Della 8.7 inch; Della Products USA, City of Industry, CA), making sure most of the fruit internal fibers were parallel as much as possible. Slices were randomly distributed in sanitized colanders and dipped in the corresponding coating solutions for 30 s. They were drained and distributed in 16-fl oz polyethylene terephthalate, unvented trays with lids (model 9057; D6, Portland, OR), 13 to 15 slices per container (450 ± 50 g), leaving minimal headspace in the container. There were four containers for each coating solution and planned storage pull-out. The control (CTRL) consisted of noncoated slices, packaged right after slicing without any water dip or rinse, as would be done in a manufacturing plant. Because of the amount of time required to process and coat the fresh-cut mangoes, physicochemical measurements and sensory evaluations were done either on the day of processing (day 0) or after overnight storage at 5 °C (day 1); subsequent measurements and evaluations were made on days 7, 11, and 14 of storage at 5 °C.

Physicochemical assessment of mango slices included firmness, color, titratable acidity (TA), soluble solids concentration (SSC), and container internal atmosphere. Measurements were taken on 10 slices per container. Firmness of fresh-cut slices was determined using a texture analyzer equipped with a 7/16-inch diameter Magness-Taylor type probe (TA-212, Stable Micro Systems) at a speed of 0.8 mm·s^{-1} and recording the force at 2.5-mm deformation (Dea et al., 2013). Slices were laid on one of the two parallel flat sides, with the probe penetrating perpendicularly to the longitudinal fibers. Color was measured with a chroma meter (model CR-400; Konica Minolta, Tokyo, Japan) used as per manufacturer's instructions. L*, a*, and b* were recorded on the center of both flat sides of each of 10 slices per container. Hue angle was calculated from a* and b* values [h° = arc tan (b*/a*)] (Plotto et al., 2010). For TA and SSC determination, samples were homogenized with distilled water (50:50, w/w) and homogenate blends were centrifuged at 15,000 g_n for 15 min (Avanti J-E centrifuge; Beckman-Coulter, Brea, CA). The supernatant (6.0 g) was titrated with 0.1 mol·L^{-1} sodium hydroxide (NaOH) to a pH 8.1 endpoint using a titrator equipped with a robotic autosampler (model 855; Metrohm, Herisau, Switzerland), a dosing interface (Dosino model 800, Metrohm) and controlling software (Tiamo v.2.5, Metrohm). SSC (percent) was determined with a digital refractometer in triplicate measurement (RX5000α; Atago, Tokyo, Japan). Headspace atmosphere in containers was determined with an oxygen and carbon dioxide (O_2/CO_2) analyzer (model 900141; Bridge Analyzers, Bedford Heights, OH) measured at storage temperature (5 °C).

Sensory evaluation of mango slices. Eight to 10 trained panelists performed descriptive sensory analysis of slices and rated the samples using a line scale with anchors at 0 (none) and 10 (high). Attributes were appearance (hue/color, cut-edge sharpness, flesh browning, moistness, and translucency), texture in mouth (firmness, juiciness, and melting), taste and mouthfeel (sweetness, sourness, bitterness, and astringency), and flavor (mango flavor, piney/terpeney, green/unripe, fermented/fizzy, and off flavor). The panelists conducted the evaluations in individual booths at 22 °C under positive pressure and red lighting. Panelists were presented with three half slices (taken from each replicate container) on plates identified with three-digit codes. The order of presentation followed a Williams' design

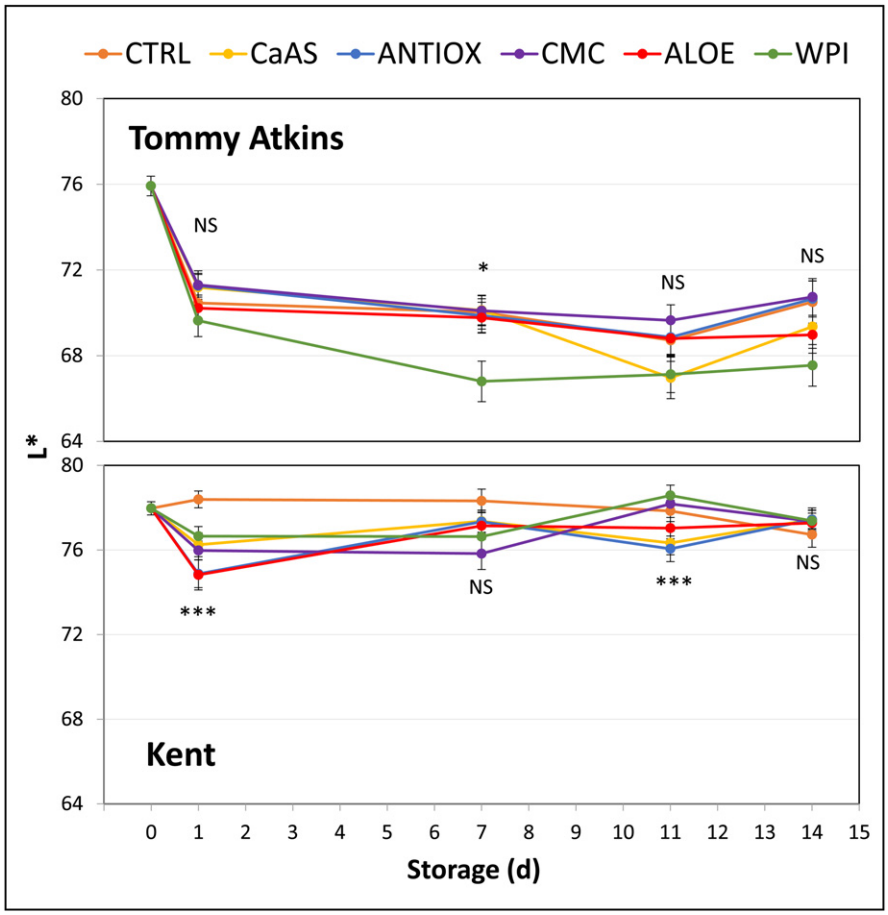

Fig. 2. Color (L*, lightness) of fresh-cut 'Tommy Atkins' and 'Kent' mango slices coated with different solutions stored at 5 °C (41.0 °F) for 14 d (CTRL = control; CaAS = calcium ascorbate; ANTIOX = antibrowning mix; CMC = carboxymethylcellulose + ANTIOX; ALOE = aloe + ANTIOX; WPI = whey protein isolate + ANTIOX). Values are means (with SE) of 10 measurements per four containers; NS = not significant between coating treatments and within a storage time; *, **, ***significant at $P < 0.05$, 0.01, or 0.001, respectively.

(Plotto et al., 2017). A recording software (Compusense Cloud; Compusense, Guelph, ON, Canada) was used to record panelist scores.

Microbial assessment of mango slices. At the end of the storage period (14 d), three to five random slices (average weight 80 g) were sampled from each container (experimental unit) before doing any other measurements to avoid external contamination. Slices were placed in sterile Whirl-Pak bags (Fisher Scientific, Pittsburgh, PA) containing 99 mL of sterile potassium phosphate buffer and agitated by an orbital shaker (Innova 2100; New Brunswick Scientific, New Brunswick, NJ) for 30 min to retrieve microorganisms from cut surfaces. Fifty microliters of buffer were plated on potato dextrose agar [PDA (Difco; BD, Sparks, MD)], orange serum agar [OSA (Difco; BD)], plate count agar [PCA (Difco; BD)], and Levine Eosin Methylene Blue agar [EMBA (Remel, Lenaxa, KS)] using a spiral plater (Eddy Jet; NeuTec Group, Farmingdale, NY). The different media types were chosen to isolate a broad range of organisms. For yeast, viable bacterial, and fecal coliform cultures, plates were incubated at 31 °C for 48 h, and results were read on a colony counter (ProtoCOL; Microbiology International, Frederick, MD). For mold enumeration, plates were further incubated at 24 °C for 7 to 10 d, and visually assessed. Colony-forming units (cfu) per milliliter were determined and adjusted for sample weight. The final results were used for mean value calculations and statistical analyses.

Statistical analysis. Instrumental data were analyzed by a two-way analysis of variance (ANOVA) with coating and storage as main effects using statistical software (SAS version 9.4; SAS Institute, Cary, NC). Duncan's multiple range tests were performed to identify significant differences between samples, within storage across coating treatments, or within coating treatment across storage. Significance was defined as $P < 0.05$. Sensory data were analyzed using statistical software (SenPAQ v. 5.01; Qi Statistics, Reading, UK). A one-way ANOVA was performed for each sensory attribute within each storage date, and for each coating across storage, using a mixed model where "panelists" are random and the main effect is tested against the interaction (panelist × sample). Fisher's least significant difference multiple comparison test was used for means separation. Microbial data (cfu/g) were analyzed by one-way ANOVA (EC treatment effect) using the statistical suite (NCSS, Kaysville, UT).

Results and discussion

Firmness. Firmness loss has a considerable impact on fresh-cut fruit acceptability and is a natural process caused by enzymatic degradation of pectin and other cell wall macromolecules (Kader, 2002; Kumar et al., 2018). Although the whole fruit firmness before processing was in the same range in both experiments, 'Tommy Atkins' slices showed a greater initial firmness than 'Kent' (Fig. 1), probably because of their higher fiber content (Abourayya et al., 2011). Fruit firmness progressively declined during storage in 'Tommy Atkins' from an initial value of 10.5 N to a final average value of 7.09 N, with no differences among samples by day 11. The sharpest decrease was found between days 1 and 7, when ALOE-coated slices maintained the highest firmness and were significantly (P = 0.031) different from CTRL and CMC- and WPI-coated slices. Similarly, 'Kent' slices softened during storage, decreasing from 7.80 N to an average of 5.47 N. Significant differences (P = 0.004) between EC treatments were found on day 11 in storage, with WPI- and M-coated slices being firmer than all other coatings, but not different

Table 2. Titratable acidity of 'Tommy Atkins' (initial: 0.55% ± 0.03%) and 'Kent' (initial: 0.55% ± 0.04%) fresh-cut mango slices coated with the different solutions and stored at 5 °C (41.0 °F) for 14 d (N = 4).

	Storage (d)				
	1	7	11	14	
Solution[z]	Titratable acidity [mean ± SE (%)][y]				$P > F$ (storage)
			Tommy Atkins		
CTRL	0.47 ± 0.03 b A[x]	0.44 ± 0.04 a A	0.49 ± 0.01 a A	0.43 ± 0.03 a A	0.400
CaAS	0.48 ± 0.04 b A	0.47 ± 0.02 a A	0.48 ± 0.03 a A	0.45 ± 0.02 a A	0.889
ANTIOX	0.55 ± 0.03 ab A	0.53 ± 0.04 a A	0.50 ± 0.03 a A	0.48 ± 0.04 a A	0.543
CMC	0.60 ± 0.02 a A	0.50 ± 0.04 a B	0.55 ± 0.02 a AB	0.46 ± 0.03 a B	0.022
ALOE	0.55 ± 0.01 ab A	0.53 ± 0.06 a A	0.50 ± 0.03 a A	0.44 ± 0.03 a A	0.168
WPI	0.62 ± 0.02 a A	0.48 ± 0.04 a B	0.50 ± 0.01 a B	0.48 ± 0.02 a B	0.007
$P > F$ (solution)	0.005	0.573	0.352	0.800	
			Kent		
CTRL	0.58 ± 0.03 a A	0.56 ± 0.02 ab AB	0.52 ± 0.02 a B	0.52 ± 0.01 aAB	0.091
CaAS	0.51 ± 0.05 a A	0.50 ± 0.03 b A	0.51 ± 0.03 a A	0.52 ± 0.04 a A	0.980
ANTIOX	0.51 ± 0.04 a A	0.59 ± 0.02 a A	0.54 ± 0.03 a A	0.52 ± 0.02 a A	0.205
CMC	0.52 ± 0.02 a A	0.57 ± 0.01 a A	0.46 ± 0.02 a B	0.55 ± 0.01 a A	0.001
ALOE	0.55 ± 0.03 a A	0.54 ± 0.03 ab A	0.49 ± 0.00 a A	0.52 ± 0.01 a A	0.225
WPI	0.49 ± 0.02 a A	0.50 ± 0.02 b A	0.48 ± 0.02 a A	0.55 ± 0.02 a A	0.426
$P > F$ (solution)	0.383	0.033	0.120	0.185	

[z]CTRL = control; CaAS = calcium ascorbate; ANTIOX = antibrowning mix; CMC = carboxymethylcellulose + ANTIOX; ALOE = aloe + ANTIOX; WPI = whey protein isolate + ANTIOX.
[y]1% = 1 g/100 mL.
[x]Within the same cultivar, values with the same lower/upper case letters within the same column/row indicate no significant differences using Duncan's multiple range test ($P > 0.05$), respectively.

from CTRL slices. Overall, statistical analysis revealed minimal differences among treatments for both varieties, and no clear pattern was found. ECs are known to prevent tissue softening by reducing water loss, whereas calcium, incorporated as calcium ascorbate or other salts, attaches to pectin, imparting strength to cell walls. This has been pointed out in a number of studies of fresh-cut mango (Plotto et al., 2010; Salinas-Roca et al., 2016) and other fruit, such as apple and cantaloupe (Koh et al., 2017; Kumar et al., 2018; Saba and Sogvar, 2016). However, in our study, neither coatings nor calcium ascorbate were effective in significantly delaying softening compared with CTRL samples. This could be because the mangoes in these tests were cut and coated at a more advanced ripeness stage than in previous studies (i.e., at the eating-ripe stage), and no treatment could prevent further softening.

Color. Decrease in L* values is frequently associated with mango flesh becoming darker, turning from yellow or light orange to dark orange, which appears overripe (Plotto et al., 2010). In 'Tommy Atkins', a sharp decrease in L* after 1 d of storage was observed, but from then on changes were negligible in most of the samples (Fig. 2). No significant differences among uncoated and coated slices were found, with the exception of WPI-coated slices, which showed significantly ($P = 0.025$) lower L* on day 7. These differences were not visually detected, neither by the researchers performing the analyses nor by sensory panelists (see sensory analysis section). In 'Kent', CTRL slices showed higher L* values than coated samples ($P < 0.0001$) on day 1, but as storage progressed, the differences became less marked and values by the end of storage were similar to initial values. There were significant differences ($P = 0.001$) in L* among coatings on day 11, with WPI being lighter than CaAS, ANTIOX, and ALOE-coated slices. This was an opposite result from 'Tommy Atkins', likely attributable to mango slice differences rather than to WPI coating.

Hue angle changes, which are indicative of color turning from light yellow to orange/red in mango fruit (Plotto et al., 2010), were calculated. 'Tommy Atkins' had lower hue angle values than 'Kent' (85.1–89.4 vs. 90.0–91.8 for 'Tommy Atkins' and 'Kent', respectively) because of its more orange flesh, but the trends observed during the course of storage were similar for both varieties. Little changes in hue angle were reported by Plotto et al. (2010) in fresh-cut mango slices coated with similar formulations; however, those authors did observe a significant effect of antioxidants in preventing L* from decreasing in comparison with control. Similarly, Robles-Sánchez et al. (2013) pointed out the effectiveness of alginate coatings combined with ascorbic and citric acids in maintaining higher L* and delaying fresh-browning. In our study, instrumental color measurements did not reveal a clear effect of the antioxidants incorporated in ECs in preventing browning. Sensory visual evaluation proved to be a more effective tool in determining differences among samples, described as follows.

SSC and TA. SSC did not show any changes between treatments nor over storage time in 'Tommy Atkins' slices, and ranged from 14.5% to 15.8%. There were some significant differences between treatments for SSC in 'Kent', with CTRL slices being higher than CaAS and ANTIOX on day 1 (16.5% vs. 15.5%) and higher that all other coated treatments on day 11 (16.0% vs. 14.9% to 15.3%). A slight decrease in SSC-coated slices (CMC, ALOE, and WPI) was observed during storage (data not shown). All treatments containing citric acid had higher TA in 'Tommy Atkins' slices compared with CTRL and CaAS on day 1 (Table

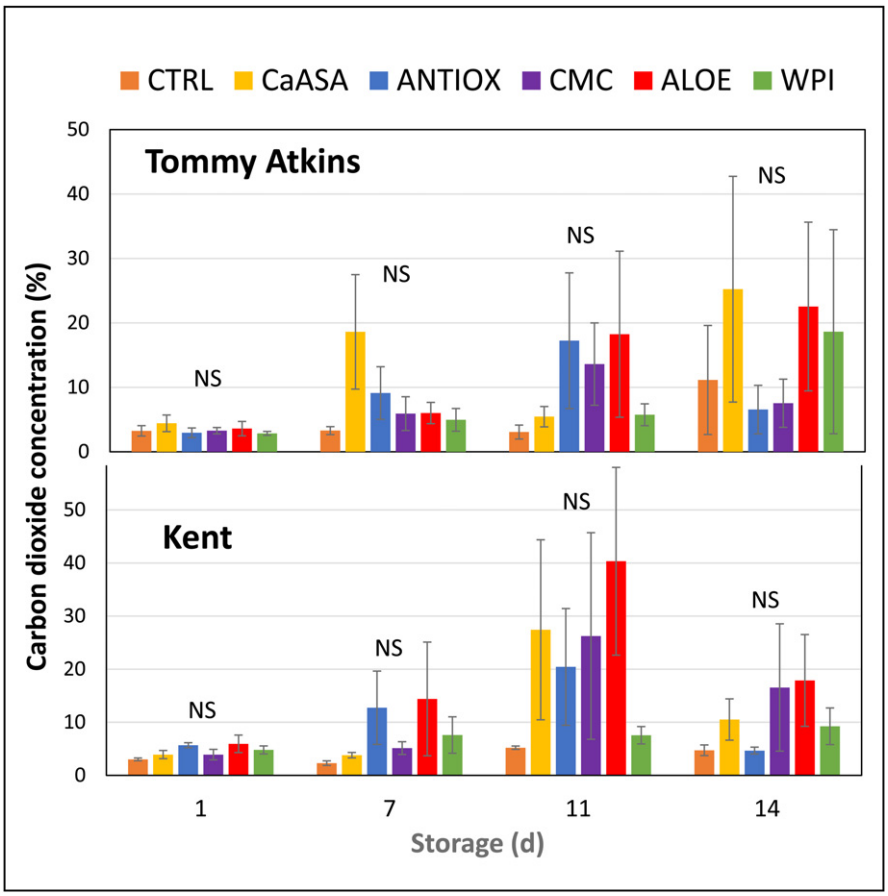

Fig. 3. Carbon dioxide (CO_2) concentration in containers with fresh-cut 'Tommy Atkins' and 'Kent' mango slices coated with different solutions stored at 5 °C (41.0 °F) for 14 d (CTRL = control; CaAS = calcium ascorbate; ANTIOX = antibrowning mix; CMC = carboxymethylcellulose + ANTIOX; ALOE = aloe + ANTIOX; WPI = whey protein isolate + ANTIOX). Values are means (with SE) of four measurements, one per container; NS = not significant between coating treatments and within a storage time.

2); however, this trend did not continue during storage, nor was it observed in 'Kent' slices. Only 'Tommy Atkins' slices coated with CMC or WPI exhibited a decrease in TA from its initial value (Table 2), and in 'Kent', CMC slices had lower TA on day 11 but not day 14, indicating little variation over time. Overall, SSC and TA levels demonstrated an eating-ripe ripeness stage of mango slices, in contrast to the previous retail store survey in which some recorded SSC values were as low as 10% and TA as high as 1.5% (Brecht et al., 2017).

Container internal atmosphere. Another function of coatings on fresh-cut fruit is reduction of respiration rate by the creation of a modified atmosphere around the product (Baldwin, 2007); however, in this study, O_2 and CO_2 in headspace of containers were highly variable (CO_2 shown in Fig. 3) and there were no differences among coatings at any of the storage times. Nevertheless, CO_2 concentration showed an increasing trend in storage for all treatments except in CTRL slices, likely due to a combination of respiration by microorganisms and mango slices (see microbial assessment). Some extreme values, up to 73% CO_2 were measured in 'Kent' stored 11 d. Values above 30% CO_2 were usually associated with O_2 of ≈0.8% to 5.0%, and a slight off odor was detected on opening those containers, which dissipated quickly. In previous studies with store-bought fresh-cut mango packaged in the same container, similar extreme atmospheres (O_2 below 1% and CO_2 over 20%) were found; the extreme atmospheres is attributed to the lack of gas exchange through the container's material (Brecht et al., 2017).

Sensory analysis. Sensory assessment plays a key role in the study of fresh-cut products because consumer buying decisions are greatly influenced by their appearance and freshness at the time of purchase (Dea et al., 2013). Objective sensory evaluations were performed after 1, 7, and 11 d of storage by a trained panel using 17 sensory attributes. With a few exceptions, significant attributes within and across storage were mostly visual: browning, cut-edge sharpness, and translucency, as well as moistness for 'Kent'.

The most obvious changes across storage were browning of slices. At the beginning of storage, little flesh browning was noted (ratings <2.0). By day 7, browning rates increased in CTRL slices, and were significantly higher than in the coated slices on day 11 for both 'Tommy Atkins' (4.3 ± 0.8) and 'Kent' (3.8 ± 0.8) (Table 3). Ratings above 5.0 would not be acceptable. Generally, browning ratings were higher for 'Tommy Atkins' than 'Kent' slices (Table 3, Figs. 4 and 5). In 'Tommy Atkins', aqueous treatments (CTRL, CaAS, and ANTIOX) showed increased browning over storage, whereas coating treatments with film formers (CMC, ALOE, and WPI) exhibited no such difference (Table 3). Moreover, 'Tommy Atkins' CaAs-treated slices, which did not include citric acid or *N*-acetyl-L-cysteine, had scores close to 5.0 after 7 d of storage. Browning remained low (less than 2.0) for 'Kent' coated slices, even after 11 d, except for CTRL (Table 3). Hence, although instrumental measurements did not show differences in L*, panelists could perceive the darker slices under white light. The mixture of antioxidants was generally effective in preventing flesh browning, both alone and in combination with ECs. Solutions of the individual antibrowning ingredients were applied to slices under the same conditions in a preliminary study and flesh browning was visually evaluated (data not shown). Citric acid was the ingredient showing the best performance in maintaining lighter color, probably through inactivation of polyphenol oxidase by both lowering pH and chelating copper at the active site of the enzyme (He and Luo, 2007). It should be noted that browning of

Table 3. Visual browning of 'Tommy Atkins' and 'Kent' fresh-cut mango slices coated with different solutions and stored 1, 7, and 11 d at 5 °C (41.0 °F) (n = 8).

	Storage (d)			
	1	7	11	
Solution[z]	Visual browning [mean ± SE (0–10 scale)][y]			$P > F$ (storage)
		Tommy Atkins		
CTRL	1.6 ± 1.0 a B[x]	4.1 ± 1.0 a AB	4.3 ± 0.8 a A	0.074
CaAS	0.4 ± 0.3 a B	4.8 ± 0.9 a A	4.3 ± 1.2 a A	0.011
ANTIOX	0.1 ± 0.1 a B	1.2 ± 0.6 b AB	1.3 ± 0.6 b A	0.076
CMC	0.6 ± 0.3 a A	1.1 ± 0.7 b A	1.1 ± 0.7 b A	0.548
ALOE	1.1 ± 0.7 a A	1.4 ± 0.6 b A	1.6 ± 0.7 b A	0.566
WPI	1.6 ± 0.7 a A	1.2 ± 0.6 b A	0.6 ± 0.3 b A	0.467
$P > F$ (solution)	0.097	<0.0001	<0.0001	
		Kent		
CTRL	0.9 ± 0.5 a B	2.2 ± 1.0 a B	3.8 ± 0.8 a A	0.004
CaAS	1.1 ± 0.7 a A	1.0 ± 0.5 b A	2.0 ± 0.7 b A	0.171
ANTIOX	0.9 ± 0.6 a A	0.7 ± 0.4 b A	1.1 ± 0.6 b A	0.371
CMC	0.5 ± 0.4 a B	1.0 ± 0.6 b B	1.3 ± 0.6 b A	0.011
ALOE	0.9 ± 0.6 a A	0. 8 ± 0.4 b A	1.2 ± 0.5 b A	0.479
WPI	0.5 ± 0.5 a A	0. 9 ± 0.5 b A	1.4 ± 0.6 b A	0.097
$P > F$ (solution)	0.226	0.028	0.000	

[z]CTRL = control; CaAS = calcium ascorbate; ANTIOX = antibrowning mix; CMC = carboxymethylcellulose + ANTIOX; ALOE = aloe + ANTIOX; WPI = whey protein isolate + ANTIOX.
[y]Values above 5.0 are not acceptable.
[x]Within the same cultivar, values with the same lower/upper case letters within the same column/row indicate no significant differences using least significant difference test ($P > 0.05$), respectively.

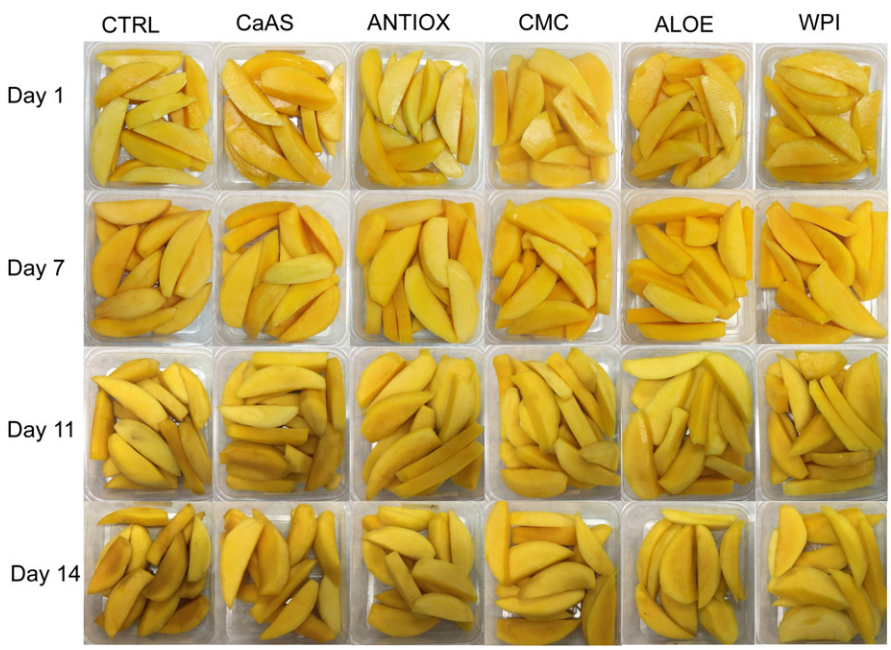

Fig. 4. Fresh-cut 'Tommy Atkins' mango slices coated with different solutions and stored at 5 °C (41.0 °F) up to 14 d (CTRL = control; CaAS = calcium ascorbate; ANTIOX = antibrowning mix; CMC = carboxymethylcellulose + ANTIOX; ALOE = aloe + ANTIOX; WPI = whey protein isolate + ANTIOX).

mango slices occurred primarily in locations where tissues had been bruised or crushed during processing as opposed to areas that had been smoothly peeled or sliced. Instrumental color measurements were taken at the center of the cut surface of a slice and this may explain why sensory panelists were able to perceive browning that was not detected by measured L* values.

Cut-edge sharpness ratings indicate the extent to which the corners of slices remain sharp or if tissue disintegration has occurred. Cut-edge sharpness of CTRL slices was significantly lower ($P < 0.05$) than that of the coated samples, right after cutting in 'Tommy Atkins' (5.1 vs. 7.1–7.9) and after 11 d in storage in 'Kent' (4.3 vs. 6.1–7.2). 'Kent' slices dipped in CaAS had the highest cut-edge sharpness after 11 d (7.2 ± 0.7), suggesting CaAS maintained tissue cohesiveness in that cultivar.

Translucency is a physiological disorder caused by loss of cellular membrane integrity that results in leakage of liquid cell contents and accumulation of liquid in intercellular spaces (Tijskens et al., 2018). The effect of coating treatments on translucency was only perceived in 'Tommy Atkins' after 7 d in storage, with ANTIOX, CMC, and ALOE having significantly fewer translucent slices (1.6 ± 0.6 to 2.0 ± 0.7) than CTRL and CaAS (3.4 ± 1.0). Overall, translucency scores did not exceed 3.5 in both cultivars, being in an acceptable range.

Other appearance attributes such as overall hue and slice moistness were also rated. For hue assessment, 0 was considered to be pale yellow and 10 to be orange. In line with the instrumental results, 'Tommy Atkins' samples were rated as more orange (5.9 to 7.3) than 'Kent' (4.6 to 6.6), and hue values remained considerably stable over the storage period with no significant differences between coating treatments (data not shown). Uncoated fresh-cut slices were expected to show signs of dehydration at the end of the storage period; moistness ratings were between 5.1 and 7.8, and between 4.0 and 6.8 for 'Tommy Atkins' and 'Kent', respectively, without significant differences between treatments. 'Kent' CMC-coated slices were the only ones exhibiting a decrease in moistness rating during storage, from 7.2 initially to 4.3 after 11 d of storage ($P = 0.003$).

Texture attributes. Firmness, juiciness, and melting were not significantly different between coatings or across storage durations for either 'Tommy Atkins' or 'Kent' ($P > 0.05$) (data not shown). Therefore, coating solutions did not affect texture in terms of mouthfeel attributes, confirming the lack of trend obtained with instrumental firmness measurements.

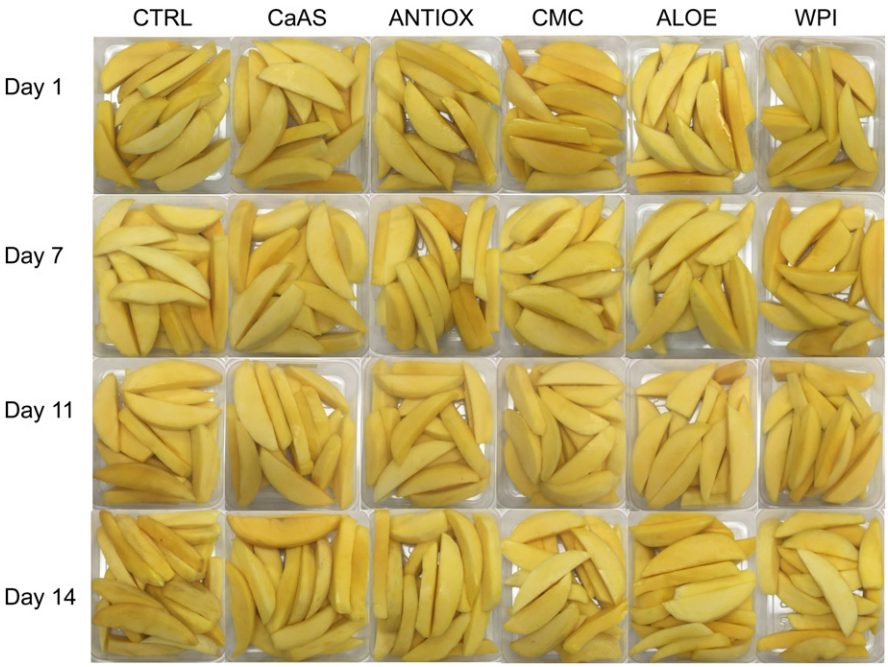

Fig. 5. Fresh-cut 'Kent' mango slices coated with different solutions and stored at 5 °C (41.0 °F) up to 14 d (CTRL = control; CaAS = calcium ascorbate; ANTIOX = antibrowning mix; CMC = carboxymethylcellulose + ANTIOX; ALOE = aloe + ANTIOX; WPI = whey protein isolate + ANTIOX).

When applying coating solutions to fresh-cut produce, one should consider not only the absence of foreign odors/taste from the ingredients, but also the formulation should not modify the product typical taste and flavor. This study showed no effect of the applied coatings on taste or flavor of fresh-cut mango slices. However, a slight decrease in sourness [3.8 to 2.3 (P = 0.039)] was detected in 'Kent' after 11 d for CMC-coated slices.

'Tommy Akins' slices stored for 1 d had higher piney/terpeney flavor (3.5 to 5.0) than 'Kent' slices (1.9 to 3.1). Piney flavor decreased in stored ANTIOX-treated slices for 'Kent' (P = 0.031), with ratings as low as 1.0 after 11 d. Scores for green/unripe and off flavor were low, suggesting that the initial firmness was appropriate for processing the fruit and that the solutions and coatings did not confer any abnormal flavor. Overripe fresh-cut mango has been reported to have an off odor (Dea et al., 2013). Ratings for fermented flavor were low too (data not shown), indicating that no fermentative metabolism took place, in spite of extreme atmospheres in a few containers.

Microbial assessment. No statistical differences were found between EC treatments in either 'Tommy Atkins' or 'Kent' slices at the end of the 14-d storage period, indicating that neither the antioxidant solutions nor coatings significantly promoted or inhibited microbial growth relative to CTRL. In general, microbial contamination was low in all samples, because in no case were counts higher than 7.7 $\times 10^4$ cfu/g. Counts were slightly higher in 'Kent' with values ranging from 6.9×10^3 to 5.2×10^4 cfu/g of total viable bacteria and from 8.4×10^3 to 7.7×10^4 cfu/g of aciduric and putrefactive microorganisms. Microbial loads in 'Tommy Atkins' ranged between 7.1×10^2 and 1.1×10^4 cfu/g for total viable bacteria and between 5.7×10^2 and 3.7×10^3 cfu/g for aciduric and putrefactive microorganisms. These results suggest that any perceived differences in flavor between treatments were not due to the presence or activity of microorganisms; however, these levels of microbial growth could account for the increased in CO_2 production after 11 and 14 d of storage (Fig. 3).

In this study, our efforts to process mangoes at an eating-ripe stage (35 N by compression firmness) were difficult to achieve because of the large variation in fruit ripeness on arrival. Mangoes were obtained directly from the importer, as would a fresh-cut processor. Nevertheless, by adjusting ripening temperature to fruit firmness on arrival, the range of whole fruit firmness before cutting was narrowed down to 30 to 50 N, with average of 43 N for 'Tommy Atkins', and 38 N for 'Kent'. Furthermore, mangoes ripen progressively from the innermost mesocarp toward the outermost tissue and often exhibit significant firmness difference between the two sides of the fruit. Thus, there was substantial slice-to-slice variability in our samples, explaining the lack of significant differences between treatments for many parameters.

'Tommy Atkins' responded to antioxidant solutions much more readily than 'Kent' by showing a reduction in slice browning. 'Tommy Atkins' slices treated with the antioxidant solution had a shelf life of 10 d in comparison with uncoated slices (less than 7 d), whereas 'Kent' slices were extended to 11 d. ECs did not add much improvement to the antioxidant solution, but they did not substantially modify fruit taste and flavor and their influence on the rest of the examined parameters was minimal.

In conclusion, antioxidants, specifically citric acid, were beneficial at maintaining the visual appearance of fresh-cut mango slices for up to 11 d, and could be recommended to processors as a simple strategy to allow marketing of ripe fresh-cut mango.

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Provoking Behavior Change In Residential Fertilizer Users

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From September 2018 to May 2019 the Florida-Friendly Landscaping team at the Seminole County Extension Office presented Fertilizer Workshops. These workshops targeted homeowners and homeowners associations, and educated participants on Best Management Practices (BMP's) for residential landscapes. Program participants also received a free bag of fertilizer. By way of mass media and 27 classes, a total of 17,454 people were educated about fertilizer BMP's. Of those, 288 people completed reflective post-surveys which revealed 97.2% increased their knowledge on the impacts stormwater (including fertilizer run-off) has on local waterbodies, 98.8% intended to use the information to fertilize their yard appropriately, and 95.3% were more confident they could fertilize appropriately. In a 6 month follow up survey, 86.1% of 129 participants reported they were currently using BMP's or had recommended BMP's to their landscaper as a result of the fertilizer workshop. These educational efforts resulted in data that shows significant behavior changes which seeks to reduce local levels of nitrogen and phosphorous, pollutants that lead to harmful algae blooms and impairments. Participants better understand sources of water contamination resulting from fertilizer misuse and have acted to change those behaviors.

When the Florida Department of Environmental Protection (FDEP) required counties to update their fertilizer ordinances, a political tug-of-war ensued, involving counties, environmental groups, professional landscapers, residents and University of Florida Institute of Food and Agricultural Science (UF/IFAS) Extension. In many counties, divisions were made, and sides were taken, but in Seminole County, the extension agent worked to find the commonalities of those involved using the science-based Florida-Friendly Landscape (FFL) principles. After the passing of the ordinance in Seminole County, the FFL program partnered with the County Watershed Division and secured a FDEP grant for $150,000. The grant funded a Fertilizer Education Program ran under the direction of the extension agent and the FFL program. The grant funded a program assistant who reported to the extension agent and they presented workshops to attain the following objectives:

1) 80% of participants will report an increase in knowledge of at least 1 of the 9 FFL principles following a program activity as measured by retrospective pre/post-survey.
2) 75.0% of participants will report the intention to adopt at least one FFL related practice following a program activity as measured by retrospective pre/post-survey.
3) 50% of participants will report adopting at least one FFL practice as measured by annual follow-up survey

Many horticulture and FFL extension agents in central Florida have a background in horticulture and have spoken with the Seminole County extension agent about her fertilizer program and other aspects of water. In a recent Central Florida Water Initiative meeting, the Seminole County extension agent's decade plus experience with that organization proved essential in providing historical insight and perspective moving forward. The agent's background, experience and programming have propelled her as a leader in this discipline.

The classes, particularly the fertilizer workshops, have been a great success as indicated by surpassing the original goals and objectives. For knowledge gained, 732 respondents reported an average attainment of 97.1%! Not only are people learning when they come to the workshops, but they are inspired to change; an average 74% of 732 respondents reported they intended to change their behaviors related to the Florida-Friendly practices. An average of 75.1% of people who attended the classes and responded to a follow-up survey (238 respondents) are changing their behaviors; for example:

- 99.0% (95/96) avoided blowing leaves and/or grass clipping down the storm-drain or onto pavement.
- 90.6% (77/85) avoided the application of phosphorous without a soil test.
- 86.4% (114/132) are using a slow-release nitrogen product.

There is currently no UF/IFAS recommended way to quantify impacts for the behaviors listed above, but we do know that they affect water quality (Reisinger et al, 2020). Because of this, the extension agent has prompted a discussion among UF/IFAS extension specialist faculty and colleagues from the FDEP to come together to work on setting some standards, which align with the water portion of the road map initiatives. We are currently working on quantifying how much nutrient run-off would be mitigated if people were to adopt specific FFL behaviors. By the end of the summer, I hope to be able to numerically quantify the impacts of these classes. For now, just know that the classes have benefited local waterways and saved the Seminole County money that would be used to treat the algal and vegetative response the nutrients bring.

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Success Story

In March 2019, a program participant named Jim attended a Fertilizing Effectively in Sandy Florida Soils workshop. Prior to coming Jim never used slow release nitrogen (SRN) products, would purchase weed n' feed and apply it to his landscape a few times a year without knowing when or how much to use. His home is in close proximity to a waterbody, but he never thought much of it. He was having patchiness and death issues with his St. Augustine grass when he saw our free class advertised in the Orlando Sentinel, a local newspaper. After attending the workshop, he followed up by discussing the results of his soil test with the extension agent. When reviewing the soil test data, he realized that the soil pH was very low and it could benefit from an addition of lime. Jim applied lime to the front yard and then, in April, applied the SRN fertilizer. These few actions taken by Jim might seem small, however when considering the impacts to his local waterbody they are huge. Not only did Jim test and alter his soil so the sod would be better equipped to absorb the nutrients he was providing, he used the right product at the right time! If Jim continues to monitor his soil every three years and use SRN products, he will be benefitting the waterways and taxpayers of Seminole County for years to come. It is possible that Jim has adopted other positive best management practices, but this alone is significant. Jim is one of 1629 people that attended a Fertilizer Workshop or a general FFL class in 2019 to learn about how their yard practices impact their environment and the local economy. Follow-up survey data results are exceptionally positive and show that Jim is not alone. As stated above, 75% of class participants reported they, like Jim, had adopted at least one FFL behavior. With more people within Seminole County adopting BMPs, there will be less nitrogen and phosphorous entering our waterways, less water quality impairments and less money spent by the county and state to remediate the vast number of waterbodies within the county.

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Shifting Tides: Lessons Learned from Virtual Plant Clinics

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Additional index words. Master Gardener volunteer, plant clinic, virtual education

Monroe County contains an archipelago of islands, which extend more than 110 miles offshore from the mainland of Florida. There is only one main coastal highway, the Overseas Highway (U.S. Route 1) that connects all of the islands from Key Largo to Key West. Traveling from the southernmost part of the county, where the main extension office is located, to the northern limits can take up to three hours. Due to these geographical restrictions, shifting programming efforts and plant clinics online in response to COVID-19 has presented new opportunities and opened extension's educational opportunities to a new and diverse audience. Through clientele feedback, we have been able to adapt our services and continue to see increases in participation for online events.

The goal of our virtual plant clinics was to create a platform where Master Gardener volunteers and the extension agent were still able to address our clientele's gardening and landscaping questions and maintain a presence in the community during COVID-19 restrictions, which halted volunteer opportunities and in-person plant clinics.

Materials and Methods

Prior to COVID-19, plant clinics were typically held seven times a month throughout Monroe County at extension offices and other off-site locations. This helped to maximize our availability in the community and offer an easy and informal way of answering any plant questions and concerns through one-on-one consultations between Master Gardener volunteers and Monroe County residents. When these opportunities were restricted, it greatly reduced our reach within the community.

The Environmental Horticulture extension agent and Master Gardener Volunteers started conducting one-hour, online plant clinics every week via Zoom in Apr. 2020. Prior to holding our first online plant clinic, the agent and volunteers met virtually via Zoom for a total of ten hours to strategize and familiarize ourselves with this new platform for programming. When we first started, it was simply an open, virtual space to meet and answer questions, much like the in-person plant clinics. However, we quickly realized the potential for attracting a wider audience and added an educational component to each plant clinic. Starting in July, the format for the plant clinics was modified to include an educational component. Each session began with a 20-min presentation on a topical gardening issue (scales in the landscape, managing plant pests with soaps, fertilizing palms, etc.) then was opened to group discussion for answering clientele questions. The new format was called Plant Clinics Plus. Program participants were also encouraged to send any questions with pictures to the Master Gardener email address so they could be addressed during the Zoom plant clinic.

In addition to our virtual plant clinics, we also established a dedicated Master Gardener email address and developed a Master Gardener Microsoft Teams page for keeping track of incoming questions and responses, storing fact sheets and documents for quick access, and as a way for Master Gardeners to interact and engage with each other through the chat function within the software.

Results

Thirty four participants attended our ten weekly plant clinics from April through June where we created an open, virtual space for answering resident's plant questions and concerns. Though participation was low, 40% of the participants had never attended previous in-person plant clinics or other extension programming and the other 60% had not engaged with extension within the last 3 years. From July through October, we held 17 virtual Plant Clinics Plus sessions, and a total of 156 participants attended these modified plant clinics, which included 65 Master Gardener Volunteers.

The new format also became a tool for introducing Florida Friendly Landscaping principles and to offer sustainable solutions for managing pests in the landscape and to introduce residents to a wider plant palette, including native plants. While 90% reported knowledge gained through the educational component of the new plant clinic format, 30% also indicated the adoption of at least one Florida-Friendly, sustainable landscaping practice which included proper identification, reducing pesticide use, and using more environmentally friendly options for controlling pests.

In addition, 30% of the Master Gardener Volunteers that participated in this new online format, had not previously volunteered at plant clinics due challenges getting to the various physical locations throughout the county and a feeling of unease answering residents' questions. One hundred percent of Master Gardener Volunteers that attended the online plant clinics reported knowledge gained and increased confidence in responding to clientele plant questions.

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Conclusion

Even though many residents that had previously engaged in our in-person plant clinics did not participate in the new online plant clinics, we were able to greatly expand our reach to a wider audience that had not previously attended any extension programming. Also, adding an educational component to each of the plant clinic sessions, received a lot of positive feedback from residents and Master Gardeners alike and increased participation in the program substantially. Moving forward, as we move back into in-person programming, we will find ways to offer hybrid programs, like our plant clinics, to engage a wider audience.

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Selecting Preemergent Herbicides for Use In Container-grown Amaryllis

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A two-part study was conducted at the nursery of Sweet Dream Amaryllis, in Valrico, Florida, to identify potential preemergent herbicides for use in the container production of landscape amaryllis (*Hippeastrum* spp.) bulbs. An initial trial of preemergent herbicides with labels allowing general use was first conducted and evaluated for visual plant toxicity and damage. Herbicides used in this study were: Corral® 2.68G (pendimethalin), Freehand® 1.75G (dimethenamid-P and pendimethalin), Snapshot® 2.5TG (trifluralin and isoxaben), Tower® (dimethenamid-P), Surflan® (oryzalin) OH2® (oxyfluorfen and pendimethalin), O-O Herbicide® (oxyfluorfen and oxidiazon), Jewel™ (oxadiazon and pendimethalin), and Rout® (oxyfluorfen and oryzalin), and an untreated control. Two additional secondary studies were conducted over six-month time to evaluate herbicides that were deemed "safe for use" on the reproductive potential of propagative bulbil or "pup" formation numbers. These herbicides were: Corral® 2.68G, Freehand® 1.75G, Snapshot® 2.5TG, Tower® and compared to an untreated check. Bulbil counts were made at six months after the treatments were applied.

Amaryllis (*Hippeastrum* spp.) are an evergreen, tropical to subtropical (USDA Zone 8-11) plant, indigenous to South America, primarily from Brazil and the Andes mountains in Peru, Argentina, and Bolivia but some species extend to the West Indies and Mexico. They were exported to Europe and the first crosses were produced in England in 1799. In the US, there is limited production, mainly in California, Texas, and Florida, which was once a major producer of bulbs (Bell, 1973). Hobbyists, enthusiasts, and collectors dominate the niche trade currently in Florida. The University of Florida also had a breeding program and released three varieties named 'Rio', 'Sampa', and 'Bahia' (Meerow, 2000). Amaryllis are primarily sold as a potted bulbs for forcing, however, they make a good addition to the Florida landscape with minimal horticultural effort. In the opinion of the authors, we are somewhat disappointed that they are not more commonplace within the landscape. The main pests are a few chewing insects (eg. grasshoppers, caterpillars), a weevil (amaryllis weevil), mites, a fungus (red scorch (*Stagonospora curtisii*)), and virus.

Propagation can be accomplished by dividing the base of the bulb. Another way is to split daughter plants or bulbils. The bulbils are then grown in either nursery containers or in common beds and may be further increased in numbers as they age and form bulbils or "pups" of their own. Usually one to three bulbils may form per year.

One of the largest problems for container production may be controlling weeds. With bulbs growing for multiple years in a container it can quickly develop a crop of weeds that will compete and choke out the growing bulbs. Hand labor is used to remove weeds but if there are a large number of containers in production the problem can quickly become economically costly.

Ornamental container growers typically use preemergent herbicides along with hand weeding to remove and reduce the number of germinating weeds on the surface of containers. With minor crops such as amaryllis there is minimal information on compatible preemergent herbicides that can be safely used to prevent weeds. In order to economically and effectively control weeds in amaryllis and educate growers and landscapers with this information, a preemergent herbicide trial was conducted.

Materials and Methods

Herbicide Visual Damage Trial. An initial trial of preemergent herbicides with labels allowing general use on ornamental plants was first conducted. The highest labeled rates were doubled to prevent over-application injury for growers. One-year old bulbs of the variety 'Susan Slade' were potted at three plants per #3 nursery container (10 inches in diameter) in 75% soil filled pots. Potting soil contained 50% compost and 50% shredded tree fines and slow release fertilizer added to the containers. Irrigation was provided by overhead sprinklers. The experiment was started in late March 2017. There were eight herbicide treatments and rates were: Corral® 2.68G (pendimethalin, 228 lbs/ac), Freehand® 1.75G (dimethenamid-P and pendimethalin, 400 lbs/acre), Snapshot® 2.5TG (trifluralin and isoxaben, 400 lbs/acre), Tower® (dimethenamid-P, 64 oz/acre), OH2® (oxyfluorfen and pendimethalin, 200 lbs/acre), Regal O-O Herbicide® (oxyfluorfen and oxadiazon, 200 lbs/ac), Jewel™ (oxadiazon and pendimethalin, 200 lbs/ac), and Rout® (oxyfluorfen and oryzalin, 200 lbs/ac), and an untreated control. Treatments were replicated three times with a nursery container being the treatment unit. Liquid herbicides were applied with a spray bottle and granular herbicides

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delivered with a shaker can. All herbicides were applied over the top of three pots and replicated under the canopy of three pots for each treatment to determine if there was a difference. Plant leaves were evaluated for visual herbicide toxicity and damage four weeks after treatment. Herbicide injury could show up in slower growth rates, longer term growth, and root damage but these were not evaluated.

Bulbil Formation Trial. A second experiment was undertaken to test if herbicides considered safe from the initial experiment would reduce future propagative potential or offset bulbil (pup) formation. Plants were grown in a similar manner as in the first experiment. The experiment was a complete randomized design with six treatments using the cultivar 'Susan Slade'. Six replications of #3 nursery containers containing three bulbs each were treated on 22 Feb. 2018. All treatments were watered in for 10 min with overhead irrigation. Treatments and rates were 1) Corral 2.68G (pendimethalin) 114 lbs/ac; 2) Freehand 1.75G (dimethenamid-P and pendimethalin) 200 lbs/ac; 3) Snapshot 2.5TG (trifluralin and isoxaben) 200 lbs/ac; 4) Tower (dimethenamid-P) 21 mL/1000 ft2; 5) Surflan (orazalyin) 3 oz/1000 ft2 and 6), and an untreated control. Surflan was added to this trial although it had not been included in the first experiment. Herbicides were applied in a similar manner as in the first experiment. Bulbil counts were taken four months after herbicide treatment (MAT) and compared to the control.

An additional experiment was conducted with three additional varieties of amaryllis: 'Sitting Bull', 'Queen of the Nile', and 'Denise'. This experiment was initiated in Apr. 2019 and finished six months later.

Results and Discussion

Herbicide Visual Damage Trial. Three weeks after treatment, herbicides that appeared to look relatively safe were Corral, FreeHand, Tower, Snapshot. Herbicides that caused phytotoxic injury were OH2, O-O Herbicide, Jewel, and Rout. Almost all damage was in the axils of the leaves right at the top of the bulb (Fig.1). There was less injury when herbicides were applied under the canopy, but no treatment was 100% injury free if it caused damage when applied overhead. It would be extremely difficult to apply these herbicides under the leaf canopy if there were any number of containers to be treated and there would be no way to guarantee the application would be only to the soil.

Bulbil Formation Experiment 1. There were no statistically significant differences between herbicide treatments and the untreated control with the cultivar 'Susan Slade'(Fig. 1). The mean values ranged from lowest, Tower with 0.22 new bulbils formed to the highest, Surflan treatment of 0.78 new bulbils. The entire experiment had a mean value of 0.5 new bulbils formed for all treatments. Due to the short, 4 month, duration of the experiment and the application to only one cultivar, an additional experiment was conducted to determine if other cultivars would respond similarly and if differences would emerge over a longer time period.

Bulbil Formation Experiment 2. The results for experiment 2 indicated that herbicides did not influence number of bulbils formed. However, there was a significant difference attributable to cultivar, in that a very small number of bulbils formed in the 'Sitting Bull' cultivar during this experiment (Table 1). Mean values of bulbils formed ranged from 0.22 with the Corral treatment to 0.88 with both the Tower and the Snapshot treatments. The authors feel that a six-month time period after herbicide treatment would be sufficient to determine if the herbicide applications would be detrimental to bulbil formation. Most preemergent herbicides loose efficacy in about 30 days in typical outdoor Florida production areas, especially with overhead irrigation. However, not all cultivars may have the same reaction and caution may be warranted before applying herbicide across different cultivars.

We did observe that 'Sitting Bull' did not form many bulbils in this experiment. We believe that this might be due to environmental factors and not herbicide interactions. Typically, 'Sitting Bull' is a strong bulbil producer.

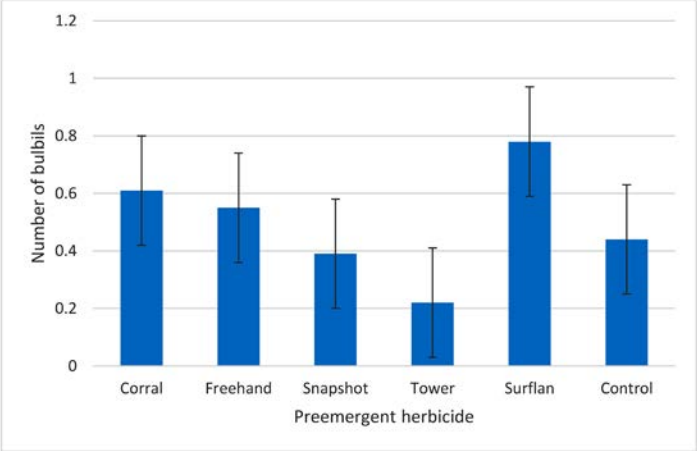

Fig. 1. Mean number of bulbils from *Hippeastrum* spp. 'Susan Slade' amaryllis grown in #3 nursery containers produced four months after treatment as affected by different preemergence herbicides. Error bars represent pooled standard deviation for experiment.

Table 1. Mean number of bulbils produced from *Hippeastrum* spp. 'Denise', 'Queen of the Nile', and 'Sitting Bull' amaryllis grown in #3 nursery containers six months after treatment as affected by different preemergence herbicides.

Preemergent	Mean number of bulbils formed per container		
herbicide	'Denise'	'Queen of the Nile'	'Sitting Bull'
Corral	0.33	0.33	0
Freehand	1.33	1.17	0
Snapshot	1.0	1.33	0.16
Tower	0.5	1.33	0
Control	0.67	0.83	0
Mean[z]	0.76 A	1.00 A	0 B
TRT	NS		
Variety	***		
TRT × Variety	NS		

[z]Mean values with the same letters within a row are not significantly different at the $P = 0.05$ level (Tukey's honest significant difference test). NS, *, **, *** Nonsignificant, or significant F test at $P \leq 0.05, 0.01, 0.001$ level, respectively.

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Landscaping for Livestock

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Additional index words. Toxic plants, landscape, livestock, equine, farm management

Marion County, Florida, is known as the "horse capital of the world" with 350,000 acres of pastureland used for livestock. In addition there are yards, fence rows, and other landscaping features that are often accessible to livestock. Lack of knowledge regarding toxic plants to livestock may cause unintentional mistreatment of animals and could lead to higher costs associated with health care of those animals. The large number of equine enthusiasts that place great value on landscaping and horse health presented a prime need for extension intervention. The Marion County horticulture and livestock extension agents teamed up to provide an in-depth class to train horse owners on various landscape strategies to be both safe and aesthetic for their properties. Topics included Florida-Friendly Landscaping™ principles such as soil testing, proper irrigation, runoff prevention, and correct plant selection. Fifteen property owners attended the workshop and survey results were as follows: 100% intend to soil test prior to fertilization; 80% could identify the proper plants for their landscaping needs and 80% understood proper practices to reduce runoff, protect beneficial insects, and mulch appropriately. The self-reported approximate value of animals if saved from toxic plants as a result of better management and plant selection was estimated at $900,000.

Florida is home to many livestock enterprises, notably the Horse Capital of the World in Ocala with more than 50,000 of the state's 385,000 horses (American Horse Council, 2017). Beef cattle, small ruminants, and poultry are also livestock enterprises in the state. Poisonous plants can cause extreme economic hardships for livestock owners in death losses, treatment of ill animals, or loss of production and decreased value of animals. In order to maintain the health of livestock animals while not sacrificing the aesthetics of farms, an appropriate landscape design is vital in areas accessible to livestock.

The consumption of toxic plants can have a variety of effects on animals including: long-term effects such as poor reproductive performance, low-weight calves taking longer to reach a marketable weight, reduction in an animal's resistance to disease, or acute effects such as loss of appetite, neurological abnormalities, hallucinations, and convulsions (James et al., 1988).

Toxins are produced by some plant species as defense mechanisms against anything that could harm them including insects, pets, livestock, and people. Many plants transmit these toxins through leaves or bark when they are eaten, injured, or disturbed. In some toxic plants, every part of the plant is toxic (or contains toxins). Depending on the route of exposure (ingestion, inhalation, or absorption) the toxin can cause mild or severe symptoms (Burrows and Tyrl, 2013).

1. Major toxicity—Can cause serious illness or death. Usually due to consumption of the toxic plant part like the seed of a toxic fruit or plant leaves.
2. Minor toxicity—Can cause minor illness symptoms such as vomiting and diarrhea.
3. Major irritant—Can cause serious allergic symptoms by irritating the skin, mouth, tongue, throat, and stomach. Can result in throat swelling, difficulty breathing, and/or burning pain.
4. Skin irritation (Dermatitis)—Can cause skin rash or irritation by direct or indirect contact with juices, sap, or thorns of plants.

Whether people are new to Florida or uncertain about which landscape plants are most appropriate for this environment as well as the health of livestock, the selection of plants and designing a landscape can be a daunting task. The goals should be plants that are safe, well-adapted, have low invasion risk and are considered Florida-friendly. A list of landscape plants that are common in Central Florida, well adapted to survive in our landscapes, and pose little threat of toxicity are listed in Table 1.

When designing a landscape, it is important to consider the elements and principles of landscape design. There are a few key aspects to understand in order to create an aesthetically pleasing and functional landscape including visual elements such as color and texture, and compositional principles like repetition and proportion (Hansen, 2010). Color is the most noticeable element in a landscape and is also the most fleeting, so selection of design based purely on aesthetics can be seasonal (Hansen and Alvarez, 2010). When considering color schemes and designs, do not limit yourself to flower colors, keep in mind colors from fruit, bark, and leaves. Texture can be created by adding different sizes of plants to various areas of the landscape. Hansen et al. (2019) describe additional information on landscape design principles.

It is also imperative to implement the "right plant, right place" method prior to designing your landscape. Be mindful of a plant's needs and plant them where their needs can be met. If a plant needs full sun and they are planted under a large tree where light cannot find it, it might never reach its full potential. During the landscape design process, consideration should also be taken in creating an environmentally friendly yard. Specifically, Florida–Friendly Landscaping™ techniques should be employed to ensure conservation of soil, filtration of water runoff, maintenance of wildlife habitat, and many other environmentally sound practices. More detailed information on Florida-Friendly Landscaping™ can be found online (Anon. n.d.).

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Table 1. Florida-Friendly Landscape™ plants that are considered safe and recommended for planting around livestock.

Scientific name	Common name	Native to Florida?	USDA Plant Hardiness Zone
Ground covers			
Ajuga reptans	Bugleweed	no	8 to 9a
Arachis glabrata	Perennial peanut	no	8 to 11
Aspidistra elatior	Cast iron plant	no	8b to 11
Cyrtomium falcatum	Holly fern	no	8b to 11
Dryopteris	Autumn fern	varies	8 to 11
Dyschoriste oblongifolia	Twin flower	yes	8 to 11
Evolvulus glomeratus	Blue daze	no	9 to 11
Glandularia tampensis	Tampa vervain	yes	9 to 11
Liriope muscari	Turf lily	no	8 to 9
Mimosa strigillosa	Powderpuff	yes	8 to 11
Nephrolepis biserrata	Giant Sword fern	yes	9 to 11
Nephrolepis exaltata	Sword fern	yes	9 to 11
Ophiopogon japonicus	Mondo grass	no	8 to 11
Phyla nodiflora	Turkey tangle frog fruit	yes	8 to 11
Thelypteris kunthii	Southern shield fern	yes	8 to 11
Trachelospermum jasminoides	Confederate jasmine	no	8b to 10
Trachelospermum asiaticum	Asiatic jasmine	no	8b to 10
Small shrubs			
Carissa macrocarpa	Natal plum	no	9 to 11
Leucophyllum frutescens	Barometer bush	no	8b to 10a
Pyracantha coccinea	Firethorn	no	8 to 9
Raphiolepis spp.	Indian hawthorn	no	8 to 9
Rosa spp.	Rose	varies	8 to 11
Rosmarinus spp.	Rosemary	no	8 to 11
Russelia sarmentosa	Firecracker plant	no	8b to 11
Sabal etonia	Scrub palmetto	yes	9 to 11
Spiraea spp.	Reeve's spirea, Bridal wreath	no	8 to 9
Large shrubs			
Abelia × grandiflora	Glossy abelia	No	8 to 9
Acca sellowiana	Pineapple guava	No	8 to 11
Acrostichum danaeifolium	Leather fern	Yes	9 to 11
Aloysia virgata	Sweet almond bush	no	8 to 11
Ardisia escallonioides	Marlberry	yes	9 to 11
Bambusa spp. (Clump types)	Bamboo	no	8 to 11
Callicarpa americana	Beautyberry	yes	8 to 10
Callistemon viminalis	Bottlebrush	no	8b to 11
Camellia japonica	Camellia	no	8 to 9
Camellia sasanqua	Sasanqua camellia	no	8 to 9
Clethra alnifolia	Sweet pepperbush	yes	8 to 9
Coccoloba uvifera	Seagrape	yes	9 to 11
Crataegus spp.	Hawthorn	varies	8 to 9
Eugenia spp.	Stoppers	varies	9 to 11
Fatsia japonica	Japanese aralia	no	8 to 11
Hamelia patens	Firebush	yes	9 to 11
Hibiscus spp.	Hibiscus	yes	8 to 11
Itea virginica	Virginia sweetspire	yes	8 to 9
Jasminum mesnyi	Japanese yellow jasmine	no	8 to 10
Jasminum nitidum	Star jasmine	no	9 to 11
Loropetalum chinense	Loropetalum	no	8 to 9
Malvaviscus arboreus	Turk's cap	no	8b to 11
Myrica cerifera	Wax myrtle	yes	8 to 10
Osmanthus americanus	Wild olive	yes	8b to 9
Osmanthus fragrans	Tea olive	no	8b to 9
Philadelphus inodorus	English dogwood	yes	8 to 9a
Pittosporum tobira	Japanese pittosporum	no	8 to 11
Severinia buxifolia	Boxthorn	yes	8b to 10
Ternstroemia gymnanthera	Cleyera	no	8 to 9

(Continued)

Table 1 (Continued). Florida-Friendly Landscape™ plants that are considered safe and recommended for planting around livestock.

Scientific name	Common name	Native to Florida?	USDA Plant Hardiness Zone
Large shrubs (continued)			
Thunbergia erecta	King's mantle	no	9 to 11
Thunbergia erecta	Sparkleberry	yes	8 to 10b
Viburnum obovatum	Walter's viburnum	yes	8 to 10
Viburnum rufidulum	Rusty blackhaw	yes	8b to 9
Viburnum suspensum	Sandankwa viburnum	no	8 to 10
Small trees			
Chionanthus virginicus	Fringetree	yes	8 to 9
Magnolia × soulangiana	Saucer magnolia	no	8 to 9a
Myrcianthes fragrans	Simpson stopper	yes	9b to 11
Viburnum odoratissimum	Sweet viburnum	no	8b to 10a
Acacia farnesiana	Sweet acacia	yes	9 to 11
Medium trees			
Carpinus caroliniana	American hornbeam	yes	8 to 9a
Taxodium spp.	Baldcypress	yes	8 to 10
Lagerstroemia indica, Lagerstroemia indica × fauriei, Lagerstroemia fauriei	Crape myrtle	no	8 to 10b
Cercis canadensis	Eastern redbud	yes	8b to 9a
Betula nigra	River birch	yes	8 to 9a
Large trees			
Juniperus virginiana	Red cedar	yes	8 to 9
Magnolia grandiflora	Southern magnolia	yes	8 to 9
Liriodendron tulipifera	Tulip poplar	yes	8 to 9a
Pinus palustris	Longleaf pine	yes	8 to 9

Materials and Methods

An opportunity to develop a new extension program presented itself to the Marion County faculty when it came to providing landscape suggestions to farm owners. The horticulture extension agent and the livestock extension agent teamed up to provide expertise that covered both the plant and animal aspects of this program. A four-hour educational program was advertised to farm owners to assist them with landscape design that would be safe for their livestock. Each agent spoke on topics related to their program areas, the livestock agent on soil, pasture, and animal health and the horticulture agent on Florida-Friendly Landscape™ principles, landscape design, and integrated pest management (IPM). After the classroom lectures, a tour of the Marion County Extension Office's demonstration gardens was conducted to show first-hand some of the landscape principles that had been discussed.

Results and Discussion

Fifteen farm owners attended this educational event. Post-program surveys suggest 100% of attendees intend to soil test their properties prior to fertilization, and 80% reported they could identify landscapes that would be suitable for their needs, utilize correct IPM strategies, and understand practices to reduce runoff of nutrients from fertilizer use, respectively. Of the 15 program attendees, 12 farm visits were generated which resulted in individual impacts and long-term relationships with extension.

Conclusions

This program was a previously untapped combination of extension agent collaboration that aimed to serve a unique audience, farm owners with landscape needs. Since the inception of this program, more in-office partnerships have taken place to serve clients that might have overlapping needs in multiple program areas. Online recordings of the Landscaping for Livestock program have been offered, which opened this education beyond Marion County. The information delivered in this program was developed into a peer-reviewed publication that is currently under review. This publication will be a resource for other agents and specialists to utilize with clients on these topics.

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—Scientific Note—

Micropropagation of a Novel *Cordyline* Hybrid

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Additional index words. in vitro propagation

Cordyline is a genus of shrubs and trees native to areas of the South Pacific Ocean. There is some disagreement as to its natural distribution considering it had been an important agricultural crop prior to European contact. In addition to its agricultural uses *Cordyline* species and hybrids have been widely adopted as commercially propagated ornamental plants. In this role *Cordyline* is utilized for its often brightly variegated foliage as well as its palm like growth habit and tropical appearance. In tropical and sub-tropical regions the various cultivars are employed as landscaping plants while in more temperate regions they are widely grown as house plants. New cultivars of *Cordyline* are often obtained from sports which produce a novel form or coloration different from the original plant. In order to commercially propagate such sports a method for rapid multiplication is desirable. Plant tissue culture and in vitro propagation have been promoted for the rapid clonal propagation of many different genera of plants. The highest rates of multiplication in vitro are often achieved through methods which utilize plant callus tissue as in ideal conditions it can be rapidly and indefinitely propagated. In applying such methods to the propagation of a *Cordyline* sport there was some uncertainty as to whether the regenerated plants would retain the sport's desired characteristics or if the micropropagated plants might revert to the original characteristics. This study was undertaken to determine if a novel *Cordyline* sport could be rapidly multiplied in vitro while maintaining its novel character.

Rooted vegetative cuttings of a single novel *Cordyline* clone were obtained from a commercial plant nursery. The cuttings were used as a source of three types of explants based on the physiologic origin of the material: Unexpanded leaf sections, meristematic region sections and sub-meristematic sections. The individual explants consisted of horizontally cut, 2–4mm thick disks of tissue. Explants were initiated onto semi-solid MS media, of which each treatment was supplemented with 1 of 9 combinations consisting of an Auxin [indole acetic acid (IAA)] and cytokinin [6-Benzylaminopurine (6-BAP)]. The treatment levels were as follows: IAA (0.05, 0.1, and 0.15 mg/L) and 6-BAP (1.0, 2.5,and 5.0 mg/L). After a period of two months the cultures were evaluated in order to determine which, if any treatments had resulted in the production of shoots. Following shoot initiation, two treatments were tested to determine a method for the rooting of multiplied shoots. The tested treatments consisted of semi-solid MS media supplemented with either 0.05mg/L 6-BAP with 1.0mg/L IBA or 0.1mg/L 6-BAP with 2mg/L IBA. After a period of 30 days the treatments were evaluated based on the percentage of shoots that had rooted successfully. In the evaluation of culture initiation both the type of explant and the initiation media were found to be significant. The effect of explant type was highly significant, only meristem region explants were found to result in successfully multiplying cultures. Media composition was also found to have a significant effect. All treatments containing IAA at the level of 0.05mg/L were found to result in significantly greater rates of initiation when compared to the two higher levels of IAA treatments. The tested levels of 6-BAP were not found to have a significant effect on the rate of successful culture initiation. In the evaluation of rooting success there was no significant difference between the two treatments. Both treatments resulted in 100% rooting success. It was determined that in vitro propagated, rooted and greenhouse hardened plantlets can retain the desired characteristics of a *Cordyline* sport.

Future studies will look into optimizing multiplication rates, determining the ideal level of cytokinin for multiplication and expanding in vitro propagation to a temporary immersion bioreactor system.

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—Scientific Note—

Estimating Neonicotinoid Insecticides Related Information Effects on Consumers' Preferences for Ornamental Plants

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Additional index words. consumer attitudes, information treatment, neonicotinoids, pollinators, plant choice

Studies in social sciences have demonstrated that psychological factors such as individual attitudes, norms, perceptions, and desires are important and integral factors to an individual's choice and decision-making (e.g., Morikawa et.al, 2002). Despite increasing concerns about the potential negative impacts of neonicotinoid insecticides on pollinator health in the regulatory community, public perceptions about the use of such pest management tools are mainly unknown.

In this study, we jointly modeled consumers' attitudes toward neonicotinoids and pollinators and their influences on choices for ornamental plants grown with or without neonicotinoids. Project participants were randomly directed to a control or treatment option in an online choice experiment. Respondents in the control group received no additional information during the choice experiment. The respondents viewed pictures of annual bedding plants, in 4-inch diameter containers, and perennial plants, in 1-gallon containers, and were asked to make 16 plant choices based on their existing knowledge and beliefs about pollinators and neonicotinoids. In each choice scenario, participants selected the product they preferred from one of three options, marked plant A, plant B, or an opt-out option (i.e., I would not buy any of these plants at this time). Meanwhile, respondents in the treatment group completed the choice experiment after viewing a 3-minute video describing the "negative and deterministic impact" of neonicotinoids on pollinators.

Results showed that additional information on neonicotinoids has a significant and direct impact on respondents' attitudes. Even though ex-ante knowledge about neonicotinoids and pollinators was similar between the control and treatment groups, respondents in the treatment groups were more concerned about the effects of neonicotinoids on pollinators' health. We observed that information treatment has a significantly positive impact on consumers' preference for labels disclosing the absence of neonicotinoids relative to labels disclosing the presence of neonicotinoids. We established a causal mapping between individual attitudes and perceptions and preference for important plant attributes.

Future studies may explore the impact of information by expanding this analysis along two dimensions: variations related to information (i.e., types, framing, and sources of information) and variations related to attitudes (i.e., trust of information sources, organizations).

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—Scientific Note—

Protocol Development for Genome Size Estimation of *Begonia* Accessions

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Additional index words. flow cytometry, begonia, genome size

With over 1800 species thus far and 68 sections (Tian et al. 2018), begonia is among the top five largest plant genera, as well as the top five bedding/potting plants sold in the United States (USDA, 2019). Wax begonia (*Begonia* × *semperflorens-cultorum*) is the primary horticultural group sold in the US. Accompanying the diverse range of morphological characters in begonia is an equally diverse range of genome size and chromosome number variation. With 1C values between 0.23 pg and 1.46 pg DNA, and chromosome numbers ranging from 2n = 16 to 2n = 156 (Dewitte et al., 2009), breeding within this genus has proven to be difficult. To make matters more complex, the genome sizes are not correlated with the chromosome numbers, as there is significant variation in chromosome size as well, making chromosome count inferences based off genome size impossible.

The overall goal of this study was to optimize a protocol for efficient flow cytometry genome size determination in begonia. To date, more than 100 accessions of begonia have been collected and organized based on species, sectional classification, foliage and flower morphology, and origin. This inventory will serve as the basis for future phylogenetic, transcriptomic, physiological, and histological studies. Currently, samples gathered from collection accessions will be subjected to flow cytometer analysis and staining to determine variation in genome sizes. The results gathered from genome and chromosome characterization can be applied to future begonia breeding. This is a first step towards understanding the mechanisms behind novel breeding of begonia.

All species and accessions are stored in a low-light greenhouse in Apopka, FL. For the most accurate estimation of genome size, fresh, young leaves were collected from each sample and stored on ice until ready for use. Three replicates were used for each species or accession. Internal standards were used to accurately determine the genome size of the unknown *Begonia* species as outlined in Doležel et al. (1989) and Arumuganathan and Earle (1991). Seeds of *Glycine max*, *Raphanus sativus*, and *Pisum sativum* were obtained from the Centre of Plant Structural and Functional Genomics in Olomouc, Czech Republic.

The protocol development consisted of testing five DNA extraction buffers to determine the most reliable buffer and duration for each sample: LB01, Marie's Nuclear Isolation Buffer, TRIS-$MgCl_2$ Buffer, Woody Plant Buffer, and Sysmex Cystain PI Absolute P (Lincolnshire, IL) (Doležel and Bartos, 2005; Doležel et al., 2007).

Once samples were collected, 1mL of DNA extraction buffer and 50µL of RNase was added to approximately 50mm^2 of unknown sample and 50 mm^2 of a known standard. The samples were then chopped up simultaneously to create a mixture of cells from both samples before filtering through a 50-µm membrane into a 3.5-mL tube. After allowing the mixture to homogenize for 1 min, 50 µm of PI stain was added to the mixture and then run through a Sysmex CyFlow Ploidy Analyzer (Lincolnshire, IL).

Preliminary results indicate that the LB01 buffer yields narrower peaks and a higher count of nuclei per sample when compared to the other extraction buffers tested. One of the primary issues with estimating genome size in *Begonia* using flow cytometry is the presence of phenolic compounds in the leaves that interfere with the extraction process and subsequent staining and processing through the flow cytometer. Sample browning is also a known issue with many plant samples. To combat the sample browning LB01 buffer and Marie's Nuclear Isolation Buffer utilize β-mercaptoethanol to reduce the oxidation that occurs prior to running samples. Ensuring all samples and buffers are stored on ice also enhances the ability to yield acceptable peaks and results. Future work will utilize this protocol to determine nuclear DNA content of all accessions.

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—Scientific Note—

Equipping Brevard County's Turfgrass Industry with Sustainable Production Know-how through the Space Coast Golf and Turf Association

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Additional index words. golf, sustainability, turfgrass

Implementing sustainable turfgrass management practices is a high priority for golf course superintendents and sports turf professionals in Brevard County, an area extending 72 miles along the Atlantic Ocean on Florida's Space Coast. Found here is the Indian River Lagoon (IRL), one of the most biodiverse estuaries on the planet with a diverse array of biota whose existence depends on the quality of the water. Protection of the IRL is a high-priority initiative of county government, and educational programs addressing this need are a high-priority of University of Florida IFAS Extension. To support the initiative, a network of turfgrass management professionals in Brevard County, officially known as the Space Coast Golf and Turf Association (SCGTA), were engaged to enhance the relationship among extension and stakeholders while promoting extension education and demonstration. The broad objective of the SCGTA program is to increase the local turfgrass industry's knowledge of and stimulate the adoption of integrated management strategies to stay profitable, sustainable and stewards of their unique landscape along the Space Coast and beyond.

Specific objectives include:

1) Annually, 75% of turfgrass management professionals in Brevard County will increase their knowledge about integrated management strategies such as efficient irrigation and fertilization practices, integrated pest management (IPM) or other techniques to increase the sustainability of their production.
2) Annually, 50% of turfgrass management professionals in Brevard County will adopt at least one integrated management strategy to increase the sustainability of their production.

To reach these objectives, five in-service workshops were held from Jan. 2019 to May 2020 reaching a total of 242 participants covering diverse topics in integrated management strategies including pest and nutrient management. Likert surveys were used to measure knowledge gain and intent to adopt integrated management practices. Sixty-nine site visits were also performed to support the implementation of strategies presented at the workshops.

Of the participants surveyed (n = 205), 95% of respondents reported an increase in knowledge while 92% reported an intent to adopt a new sustainable production practice after attending one of the five workshops. Adoption of practices presented at the workshops were documented with site visits. Examples of these practices include removing grass clippings for bermudagrass mite management, soil testing for nutrient needs and fertilizer applications, rotating modes of action to manage pesticide resistance, soil flushing for mole cricket monitoring and using soil moisture sensors for irrigation management.

The program will continue to actively engage and assist turfgrass growers with implementing the best nutrient, pest and irrigation management strategies that will increase the productivity and profitability of the turf industry in Brevard County while limiting the impact on the Space Coast's abundant natural resources, such as the IRL. By fostering associations with stakeholders, horticulture extension agents can enhance their relationships with target clientele, and these associations will in turn enhance educational activities that will increase the knowledge and promote the adoption of sustainable productions practices to help the industry stay profitable, sustainable and stewards of their landscapes.

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—Scientific Note—

Multispecies Lawns: An Alternative Strategy for Water Conservation and Ecosystem Functions

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Turfgrass is the most common plant type in suburban developments with over 5 million acres of residential turf in Florida. While providing valuable aesthetic, environmental, and recreation benefits, the maintenance of typical Florida turfgrass lawns includes regular irrigation and fertilization. Moreover, turfgrass lawns have low plant diversity. Improper maintenance practices can lead to excessive and unnecessary water usage and degradation to water quality.

Increasing plant diversity in lawns with flowering native species may reduce the need for irrigation and fertilization, as these species can maintain green coverage with fewer inputs.

Furthermore, insect pollinator populations are declining worldwide. The addition of native flowering plants may enhance ecosystem services such as pest regulation, pollination, and arthropod biodiversity. Regardless of the benefits, adding native plants to lawns will only be a viable alternative if homeowners feel this approach is aesthetically appealing and manageable. The overall goal of this project was to determine the aesthetic and functional value of multispecies lawn plantings of turfgrasses, legumes, and forbs compared to bahiagrass (*Paspalum notatum*) monocultures, a low-input turfgrass species well-suited to Florida.

In Mar. 2019, eighteen 2 × 2 m plots were installed at the Discovery Gardens in Lake County, FL. Each plot was randomly assigned one of three planting levels: bahiagrass monoculture, bahiagrass-forb mixture, or forbs alone. Selected to represent a diversity of flower color, type, and height, the forb species consisted of *Salvia lyrata*, *Phyla nodiflora*, *Mimosa strigillosa*, and *Coreopsis leavenworthii* (Fig. 1).

Fig. 1.Clockwise from top left: Bahiagrass (*Paspalum notatum*) monoculture treatment, bahiagrass-forb treatment and forb alone treatment in landscape plots at Discovery Gardens in Lake County, FL.

The University of Florida IFAS Center for Land Use Efficiency funded this project. Thanks to the Lake County Master Gardener Volunteers for their time and to Lake Jem Farms for the donation of plant materials.

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Initially mowing regimes were the same among all plots in the study, but after careful observation and consideration, mowing regimes were adjusted. Bahiagrass monoculture plots were mowed once a week. The bahiagrass-forbs mixture plots were mowed every other week, while forbs plots were mowed only to reduce the reseeding of weed species. Weeds were rogued by hand and after establishment each plot received fifteen minutes of weeding monthly. Irrigation occurred twice after establishment, only during times of severe drought.

Every three months, arthropods were monitored and identified using sticky traps and pitfall traps, pollinator visitation was recorded, flowers were counted and percent plant coverage of plots was estimated. Finally, a 15-question survey was administered to various stakeholder groups to determine how important sustainable landscaping is to them and how likely they are to integrate any of these plantings into their landscapes.

As predicted, preliminary results suggest that, as abundance and diversity of flowering resources increases, so do pollinator visits and pollinator diversity. Both treatments containing forbs attracted more pollinators than bahiagrass monoculture treatments. Interestingly, no difference in ground dwelling arthropod abundance or taxonomic richness was observed among treatments.

During the summer and spring, approximately 10% greater coverage of green plant material occurred in the two treatments containing forbs compared to bahiagrass monoculture treatments. In the winter, bahiagrass monoculture treatments provided more green coverage. The arthropod and percent coverage data were collected from Summer 2019 through Spring 2020, while analysis from other periods is ongoing.

Covid-19 limited the use of stakeholder surveys, but 21 people from the local green industry completed a survey in early March. Ten percent of participants were likely to use the bahiagrass-forbs mixture as a lawn in their front and side yards, while 14% would use this blend in their backyard. Eighteen percent would use the forbs only treatment in their front yard, while 23% would use forbs as a lawn in their side and backyards. To best capture public perception of blended species lawns, diverse stakeholders must be surveyed. It is notable that this project piqued the interest of a premier wholesale tree farm which is now pursuing the development and production of alternative plant species for lawns.

Despite the observed benefits of forb treatments, weed pressure was notably greater than the bahiagrass monoculture treatments. The lack of best management practices and tools for establishing multi-species lawns to minimize weed pressure justifies additional research. Areas to further explore also include optimizing irrigation for improved aesthetics, evaluating different species combinations, and expanding the palette of suitable groundcover plant materials.

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'UF-1013-1': An Infertile Cultivar of *Lantana camara*

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Lantana (*Lantana* L., Verbenaceae) is produced and grown as an ornamental plant in the United States and many other countries in the world. The ornamental value of lantana comes from its bright-colored flowers. Other attributes of this plant include attraction to multiple species of butterflies; tolerance of drought, heat, and salt; low maintenance requirements; and ease of propagation (Bachman, 2018; Schoellhorn, 2004). With these attributes, lantana is commonly used in the landscape and gardens, including butterfly gardens and water-saving xeriscaping gardens. Propagation and production of lantana plants have been a significant component of the environmental horticulture industry in a number of countries in the world and multiple states in the United States. For example, a survey of the Florida nursery industry indicated that 19% of the responding nurseries produced lantana and the annual sales value of lantana in Florida was at more than $40 million (Wirth et al., 2004).

The majority of lantana plants in commercial production and landscape use belongs to *Lantana camara* L. This species is native to Central and South America, including the West Indies (Sanders, 2001) and was introduced to the United States in the 1800s (Swearingen and Bargeron, 2016). Since then, *L. camara* has escaped cultivation and become naturalized in 13 states in the United States (Swearingen and Bargeron, 2016). In Florida, escaped *L. camara* has hybridized with *Lantana depressa* Small, endangering this native species (FLEPPC, 2019; Hammer, 2004; Sanders, 1987). *Lantana camara* has been listed as a Category I invasive species in Florida by the Florida Exotic Pest Plant Council (FLEPPC, 2019). The University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) Assessment of Non-Native Plants in Florida's Natural Areas concluded that invasive *L. camara* is not to be recommended for production or landscape use in south, central, and north Florida (http://assessment.ifas.ufl.edu/). The assessment was based on data collected from escaped, naturalized *L. camara*. The Georgia Exotic Pest Plant Council (GA-EPPC, 2019) listed *L. camara* as a Category 3 exotic plant, which is a minor problem in Georgia natural areas, or is not yet known to be a problem in Georgia but is known to be problem in adjacent states (GA-EPPC, 2019).

A research program was initiated in 2004 at UF/IFAS's Gulf Coast Research and Education Center (GCREC) to identify and develop infertile *L. camara* cultivars. The majority of commercial *L. camara* cultivars were found to be fertile (Czarnecki, 2011). New infertile cultivars are needed by the environmental horticulture industry and for protecting native species and the environment (Bechtloff et al., 2019). In 2016, we released two infertile triploid lantana cultivars, UF-1013A-2A (Bloomify Red) and UF-1011-2 (Bloomify Rose) (Deng et al., 2017). They performed well in variety trials (Bachman, 2018) and are well received by nursery growers.

'UF-1013-1' (Figs. 1 and 2) is a sibling of 'Bloomify Red' and shares the high level of male and female infertility and superior plant performance with 'Bloomify Red', but 'UF-1013-1' has shorter plants, larger flowers, a higher nuclear DNA content, and a different molecular marker profile. On the basis of the high level of male and female infertility of 'UF-1013-1' and its lack of hybridization potential with *L. depressa*, an Infraspecific Taxon Protocol request was submitted to the UF/IFAS Invasive Plants Working Group,

Fig. 1. Plant (≈11 weeks old) and flowers of 'UF-1013-1' lantana (*L. camara*) in a container trial in Michigan (from Mr. K. Hurd, Proven Winners).

and the Working Group unanimously approved the release of 'UF-1013-1' as a new sterile cultivar.

Origin

'UF-1013-1' resulted from a cross between breeding line DROP-25 and 'Landmark Flame Improved'. The cross was made in Fall 2010 at the GCREC, and the cultivar was initially selected as an individual plant in April 2012. The intellectual property status of 'Landscape Flame Improved' is unclear. Google searches linked this cultivar to one cultivar called Balandimfla, but it was not found in the USPTO database. 'UF-1013-1' has been asexually propagated by cuttings for at least six generations, and its characteristics are stable and consistent.

Fig. 2. Plants of 'UF-1013-1' lantana (*L. camara*) grown on a mulched ground bed in Citra, FL, in full sun (May 2019).

Description

Description of color for plant parts were based on comparison with the Royal Horticultural Society Color Chart (Royal Horticultural Society, 1986). Plants used for describing color and other attributes were propagated from rooted cuttings and grown outdoors in 11.4-cm pots in the shade house in Balm, FL, for 11 to 16 weeks.

Plants of 'UF-1013-1' are multistemmed shrubs, with a mounding growth habit, ≈36 cm wide and 25 cm tall. Young stems are yellow-green [Royal Horticultural Society (RHS) 144B], square, semiwoody, and covered with soft hairs (scabrous) but no prickles; old stems are round, light brown (RHS 199A/B), and smooth, with few hairs. Leaves are opposite, simple, with petioles 1.4 to 2.7 cm long and in yellow-green (close to RHS 146C). Mature leaf blades are ovate, 5.2 to 9.4 cm long, 3.1 to 6.2 cm wide, with serrated margins and an average of 39 teeth per leaf, an obtuse to broadly truncated base, and an acute apex. The upper leaf surface is green (RHS 147A), covered with soft hairs. The lower surface is green (RHS 147B). Inflorescences are umbel-like, ≈4.5 cm wide across the top, bearing 22 to 30 flowers, and with yellow-green (RHS 144A) peduncles 1.4 to 3.3 cm long. Flowers are bright yellow (RHS 13A) when opening initially and then turn red (RHS 34A). Inflorescences rarely bear fruit.

The nuclear DNA content of 'UF-1013-1' was determined using a CyFlow Cube 6 flow cytometer (Sysmex, Partec GmbH, Otto-Hahn-Straße 32 D-48161, Münster, Germany) and the procedure described by Doležel et al. (2007) and modified by Cao et al. (2014). Pea cultivar Ctirad (*Pisum sativum*) with a nuclear content of 9.09 pg/2C was selected as the internal reference for use in this study. The nuclear DNA content of lantana samples was calculated according to Doležel et al. (2007): sample nuclear DNA content (pg/2C) = internal reference nuclear DNA content (9.09) × (mean fluorescence value of sample / mean fluorescence value of internal reference). The ploidy level of 'UF-1013-1' was determined by comparing its nuclear DNA content with the DNA content of known diploid, triploid, and tetraploid lantana cultivars. 'UF-1013-1' is a triploid with an average nuclear DNA content of 4.82 pg/2C (Table 1).

'UF-1013-1' was analyzed for DNA fingerprint or molecular marker profile using three lantana-specific simple sequence repeat (SSR) primers. Lantana genomic DNA was isolated from lantana leaves at the GCREC in Balm, FL. Primers were developed as described by Gong and Deng (2011) from SSR-enriched lantana genomic sequences (L. Gong and Z. Deng, unpublished data; Table 2). Polymerase chain reaction (PCR) amplification, capillary electrophoresis, and allele scoring were performed at the USDA/ARS Fruit and Tree Nut Research Laboratory, Byron, GA, using a procedure previously described by Chen et al. (2014) with minor modifications. PCR was performed on a C1000 Touch Thermal Cycler with a CFX384 block module (BioRad, Hercules, CA) in a 5-µL volume consisting of 1 × PCR buffer, 0.2 mM dNTPs, 2 mM $MgCl_2$, 0.3 µM of the dye-labeled forward and regular reverse primers, 0.5 units of Taq DNA polymerase (BioExpress, Kaysville, UT), and ≈10 ng lantana genomic DNA. A touchdown PCR program was used, with an initial denaturation at 94 °C for 3 min, followed by 10 cycles of denaturation at 94 °C for 30 s, annealing at 68 °C for 30 s with a 0.5 °C decrease each cycle, and extension at 72 °C

Table 1. Nuclear DNA content, ploidy level, and pollen stainability of lantana cultivar UF-1013-1 and two controls, 'Bloomify Red' and 'Pink Caprice', grown in Balm and Ft. Pierce, FL, in full sun in 2015.

			Pollen grains examined (no.)		Pollen stainability (%)[z]		
Cultivars	Nuclear DNA content ± SD (pg/2C)	Ploidy level	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Avg
UF-1013-1	4.82 ± 0.11	3*x*	1,464	1,840	2.0 b	2.4 b	2.2 b
Bloomify Red	4.54 ± 0.08	3*x*	2,122	1,466	1.5 b	4.5 b	3.0 b
Pink Caprice	6.25 ± 0.17	4*x*	1,271	1,094	70.8 a	75.3 a	73.1 a

[z]Anthers in Expt. 1 collected from plants grown in ground beds in full sun in Balm, FL; anthers in Expt. 2 collected from plants grown in field trials in Ft. Pierce, FL. Pollen stainability data were arcsine-transformed before analysis of variance was performed. Means with the same letter within the column are not significantly different by the least significant difference procedure at $P < 0.05$.

Table 2. Simple sequence repeat (SSR) marker analysis of 'UF-1013-1' and 'Bloomify Red', breeding line DROP-25 (one of the parents of 'UF-1013-1' and 'Bloomify Red'), and 'Pink Caprice', an invasive type of *L. camara*.

	Amplicons (alleles) amplified by SSR markers (bps)[y]											
	Marker Lantana11				Marker Lantana12						Lantana20	
Lantana cultivars[z]	150	152	156	160	135	143	145	147	150	152	93	109
UF-1013-1		+		+	+	+		+			+	
Bloomify Red	+	+		+		+	+				+	
DROP-25	+	+		+	+	+	+	+			+	+
Pink Caprice		+	+	+		+	+		+	+	+	+

[z]The other parent of 'UF-1013-1' ('Landmark Flame Improved') was not available for analysis. Nucleotide sequences of the three pairs of primers are as follows: lantana11F: (M13 tail sequence)-TGCAATTGGAGGCTTTTTCT and lantana11R: AAAGCAGCTTCAAGTTTGTGC; Lantana12F: (M13 tail sequence)-GGATGAGATGATAAGGTAGGGTGT and Lantana12R: TTGGTGGTGATGACTTTGATTC. Lantana20F: (M13 tail sequence)-AGAATCAGGG TTTGGGGTTG and Lantana20R: TCGTAGCCACCACTCCTCAC. The M13 tail sequence was 5′-CCCAGTCACGACGTTG-3′.
+ indicates the presence of the respective alleles in the cultivars.

Table 3. Fruit production of 'UF-1013-1' and the two control cultivars Bloomify Red and Pink Caprice grown outdoors in ground beds in full sun at two sites in Florida (2015).

Expt. Site	Cultivars	Fruit per peduncle 8 to 21 wk post transplanting (WPT)				Total peduncles examined (no.)	Total fruit collected (no.)	Total mature fruit collected (no.)	Avg fruit per peduncle
		8	12	16	21				
Balm[z]	UF-1013-1	12 Aug.	9 Sept.	7 Oct.	11 Nov.	480	2	0	0.004 b
	Bloomify Red	0.008 b[y]	0.008 b	0 b	0 b	481	11	0	0.023 b
	Pink Caprice	0.012 b	0.050 b	0.025 b	0 b	480	4,950	1,416	10.313 a
		14.258 a	8.850 a	10.117 a	8.025 a				
		8	12	17	21				
Ft. Pierce[x]	UF-1013-1	12 Aug.	10 Sept.	14 Oct.	11 Nov.				
	Bloomify Red	0.013 b	0 b	0.038 b	0 b	320	4	3	0.013 b
	Pink Caprice	0 b	0 b	0 b	0 b	320	0	0	0 b
		11.588 a	8.325 a	6.263 a	5.588 a	320	2,541	1,832	7.941 a

[z]Twenty peduncles randomly sampled on each of the six plants in three blocks in Balm, FL, over 4 months in 2015.
[y]Means with the same letter within columns within the trial site are not significantly different by the Tukey's honestly significant difference procedure at $P < 0.05$.
[x]Twenty peduncles randomly sampled on each of the four plants in four blocks in Ft. Pierce, FL, over 4 months in 2015.

for 45 s, followed by 25 more cycles with a constant annealing temperature at 63 °C (other parameters were the same), plus a final extension at 72 °C for 15 min. The dye (FAM)-labeled PCR products were separated on a 3500 Genetic Analyzer (Life Technologies, Carlsbad, CA) to generate the chromatographic trace files. The SSR allele table and peak chromatograms were generated using GeneMarker 2.4 (SoftGenetics, State College, PA). In 'UF-1013-1', two major amplicons (152 and 160 bp) were detected with the Lantana11 marker, three major amplicons (135, 143, and 147 bp) with the Lantana12 marker locus, and one major amplicon (93 bp) with the Lantana20 marker (Table 2). These amplicons are expected to represent the number of alleles and the status of heterozygosity at each marker locus.

In side-by-side field trials in Citra, FL, in 2018 and in greenhouse trials in Balm, FL, in 2020, plants of 'UF-1013-1' were shorter and its flower clusters were larger than its sibling cultivar Bloomify Red. 'UF-1013-1' also has 6.2% higher nuclear DNA content (4.82 pg/2C vs. 4.54 pg/2C in 'Bloomify Red') (Table 1). 'UF-1013-1' carries two alleles (135 and 147 bps) at the marker Lantana12 that are absent in 'Bloomify Red' and does not carry the 150-bp allele at the Lantana11 that is present in 'Bloomify Red' (Table 2).

Replicated Field Trials to Evaluate Plant Fertility and Performance

'UF-1013-1' and 'Bloomify Red' were tested in the same replicated trials (Deng et al., 2017). As reported previously (Deng et al., 2017), the two field trials were conducted at the GCREC in Balm, FL [southwest Florida, USDA hardiness zone 9a (USDA Plant Hardiness Zone Map, 2019), and AHS heat zone 10 (AHS, 1998)], and at the Indian River Research and Education Center (IRREC) in Ft. Pierce, FL (southeast Florida, USDA hardiness zone 9b, and AHS heat zone 9–10). The experimental design in the Balm trial was a randomized complete block with three blocks and two plants per plot. Ground beds at the GCREC were raised ≈20 cm, fumigated with Pic-Clor 60 (active ingredients 1,3-dichloropropene and chloropicrin) at 448 kg per hectare in Feb. 2015, and covered with white-on-black plastic. The soil was EauGallie fine sand containing ≈1% organic matter and a pH value between 6.2 and 7.4. The experimental design used in the Ft. Pierce trial was a randomized complete block with four blocks and single-plant plots. Ground beds at the IRREC were not fumigated but treated with a preemergent herbicide [Sandia, 75.0% a.i. (halosulfuron-methyl), Yuma, AZ] at a rate of 0.1056 $g \cdot L^{-1}$ and a 2% solution of glyphosate (Roundup WeatherMAX, 48.8% a.i., St. Louis, MO) and covered with black groundcover. The soil was Ankona sand with ≈2.8% organic matter, pH 5.9. At each site, 'Pink Caprice' was included as a "resident species" taxon. Also at each site, 'Bloomify Red' was included as a sterile check. In addition, 21 commercial cultivars with various levels of male and female fertility were randomly placed in each block at both sites where 'UF-1013-1' and 'Bloomify Red' were evaluated.

Before installation in the field trials, plants were propagated at GCREC. Cuttings were rooted in 128-cell Speedling trays filled with a customized potting substrate. The bottom ends of cuttings were treated with Dip'n Grow (1:9 dilution, final concentration 0.1% indole-3-butyric acid and 0.05% 1-naphthaleneacetic acid) (Dip'n Grow Inc., Clackamas, OR). Rooted cuttings were pinched on 13 Mar. 2015 and transplanted on 5 May 2015 to 10.2-cm plastic containers filled with a commercial potting mix (Fafard 3B) and grown in the greenhouse at GCREC (at 15 °C/night to 33 °C/day). The container-grown plants were transplanted to the ground beds in the week of 12 June 2015. Each plant was top-dressed with ≈15 g of a controlled-release fertilizer (Osmocote; 15N–$9P_2O_5$–$12K_2O$, 5 to 6 months, Scotts, Marysville, OH) and irrigated through a seep system at GCREC and through drip tapes, twice a week and 2 h per irrigation event, at IRREC.

Pollen Stainability

Lantana pollen stainability has been used as an indicator of lantana's male fertility (or sterility) and hybridization potential with *Lantana depressa* (Czarnecki, 2011; Czarnecki et al., 2012, 2014; Dehgan and Guy, 2004). Generally, triploid lantana cultivars with low pollen stainability (<15%) have little potential to cross-pollinate *L. depressa* (Czarnecki, 2011).

Two experiments were conducted to assess the pollen stainability of 'UF-1013-1'. In Expt. 1, newly opened flowers were collected from plants grown in Balm, FL, in late July 2015, whereas in Expt. 2, flowers were collected from lantana plants grown in Ft. Pierce, FL, in mid-Aug. 2015. In both experiments, anthers were stained with 10^{-6} M fluorescein diacetate (Sigma-Aldrich, St. Louis, MO) in 0.22 M sucrose at room temperature in the dark for 1 h, as described by Czarnecki et al. (2014) and Deng et al. (2017). Plump, round pollen grains fluorescing bright yellowish green light were considered stainable, whereas misshaped, nonfluorescing, or unevenly and lightly fluorescing pollen grains were counted as non-stainable.

The number of pollen grains examined for each lantana cultivar in each staining experiment was between 1094 and 2122 (Table 1). An analysis of variance (ANOVA) and mean separation were conducted using JMP Pro 13.2.0 (SAS Institute, Cary, NC) to compare the pollen stainability of 'UF-1013-1' with that of 'Bloomify Red' and 'Pink Caprice'. The average pollen stainability of 'UF-1013-1' was 2.2% (Table 1), comparable to the average pollen stainability of the previously released sterile cultivar Bloomify Red. The average pollen stainability of 'Pink Caprice' was 73.1%. These results indicate that

Table 4. Plant height, width, and size index of 'UF-1013-1' compared with 'Bloomify Red' and 'Pink Caprice' grown in Florida in ground beds and full sun.

		Plant ht (cm)[z]		Plant width (cm)[z]		Plant size index[z]	
Trial site	Cultivar	2 MPT	5 MPT	2 MPT	5 MPT	2 MPT	5 MPT
Balm	UF-1013-1	35.7 b[y]	47.8 b	51.6 b	86.6 b	0.076 b	0.288 b
	Bloomify Red	42.3 b	53.7 b	51.7 b	90.2 b	0.092 b	0.353 b
	Pink Caprice	59.5 a	89.3 a	103.0 a	211.7 a	0.491 a	3.168 a
Ft. Pierce	UF-1013-1	36.8 b	45.8 b	50.4 b	76.5 b	0.074 b	0.211 b
	Bloomify Red	36.3 b	42.0 b	35.8 b	76.5 b	0.049 b	0.212 b
	Pink Caprice	57.0 a	92.5 a	110.5 a	215.3 a	0.551 a	3.406 a

[z]Plant height measured from the ground level to the highest point of the plant; plant width measured in two perpendicular directions and the average of the two widths reported. Plant size index (cubic meters) calculated using the following formula: size index = plant height (cm) $\times \pi \times$ [plant width (cm) / 2)^2 / 1,000,000, where π = 3.14. 2 or 5 MPT = 2 or 5 months posttransplant.

[y]Means with the same letter within columns within the trial sites are not significantly different by the Tukey's honestly significant difference procedure at $P < 0.05$.

Table 5. Plant and flower intensity ratings of 'UF-1013-1' compared with 'Bloomify Red' and 'Pink Caprice' grown at the Gulf Coast Research and Education Center) in Balm, FL, and at the Indian River Research and Education Center in Fort Pierce, FL, in ground beds in full sun in Aug. through Nov. 2015.

		Plant rating[z]				Flower intensity rating[y]			
Trial site	Lantana cultivar	Eval. 1	Eval. 2	Eval. 3	Eval. 4	Eval. 1	Eval. 2	Eval. 3	Eval. 4
Balm	UF-1013-1	4.7 a[x]	3.8 a	3.7 ab	3.0 a	2.7 a	1.7 b	3.3 b	1.5 ab
	Bloomify Red	3.8 ab	3.8 a	4.0 a	3.3 a	2.5 a	2.5 a	4.3 a	1.0 b
	Pink Caprice	3.5 b	3.0 b	3.0 b	2.0 b	1.0 b	1.0 b	3.0 c	2.0 a
Ft. Pierce	UF-1013-1	3.5 b	3.0 ab	3.0 b	2.8 NS	3.8 NS	3.3 ab	2.8 NS	2.3 b
	Bloomify Red	3.0 b	2.0 b	3.0 b	2.8	3.0	1.8 b	2.8	2.0 b
	Pink Caprice	5.0 a	4.3 a	4.0 a	2.0	3.8	3.8 a	4.0	3.0 a

[z]Plants were rated on a scale of 1 to 5, with 1 = performing very poorly and unacceptable, 3 = performing fairly and acceptable as ornamental plants, and 5 = performing outstandingly and highly desirable. Lantana plants were transplanted to ground plants in June 2015. The four evaluations were performed in Aug. (Eval. 1), Sept. (Eval. 2), Oct. (Eval. 3), and Nov. (Eval. 4), 2015, respectively.

[y]Flower intensity was rated on a scale of 1 to 5, with 1 = 0% to 20%, 2 = 21% to 40%, 3 = 41% to 60%, 4 = 61% to 80%, and 5 = 81% to 100% of the maximum flower coverage of the plant. The four evaluations were performed in Aug. (Eval. 1), Sept. (Eval. 2), Oct. (Eval. 3), and Nov. (Eval. 4), 2015, respectively.

[x]Means with the same letter within columns within the trial sites are not significantly different by the least significant difference procedure at $P < 0.05$. NS = not significantly different by the Tukey's honestly significant difference procedure at $P < 0.05$.

the pollen stainability (or male fertility) of 'UF-1013-1' was reduced by 95% from that of 'Pink Caprice'.

Female Sterility

Fruit (seed) production per peduncle and seed germination or seedling emergence are the primary factors determining lantana's female fertility (or sterility) (Czarnecki, 2011; Czarnecki et al., 2012). A female fertility index (FFI) can be derived by multiplying fruit production per peduncle with seed germination and used to compare the female fertility (or sterility) of different lantana cultivars.

Fruit production per peduncle in replicated field trials. Data were collected from the replicated field trials in Balm and Ft. Pierce described earlier. Four data collections were made for each plant at each trial site. In each collection, 20 peduncles were randomly sampled from each plant, and drupes on these peduncles were counted as immature (green) or mature fruit. Thus, in each fruit collection, ≈120 peduncles were sampled for each cultivar trialed in Balm, and ≈80 peduncles were sampled for each cultivar trialed in Ft. Pierce. The four fruit collections in Balm were made on 17 Aug., 14 Sept., 16 Oct., and 18 Nov. 2015, respectively. The fruit collections in Ft. Pierce were conducted on 12 Aug., 10 Sept., 14 Oct., and 11 Nov. 2015, respectively. An ANOVA and separation of mean fruit production values by the Tukey's honestly significant difference procedure were performed using JMP Pro 13.2.0 (SAS Institute) to compare the fruit production of 'UF-1013-1' with that of 'Bloomify Red' and 'Pink Caprice'.

As previously reported by Deng et al. (2017), 'Pink Caprice' produced the largest number of drupes among all the entries in the two replicated trials (Table 3). Each peduncle had an average of 7.941 drupes in Ft. Pierce and 10.313 drupes in Balm, averaged to 9.127 drupes per peduncle across the two sites and four harvests. The number of drupes per peduncle for the sterile cultivar Bloomify Red ranged from 0 to 0.050 and averaged to 0.015 across the two sites over the 4 months. The number of drupes 'UF-1013-1' produced per peduncle ranged from 0 to 0.038 and averaged to 0.009 across two experimental sites and over 4 months (Table 3). This level of fruit production in 'UF-1013-1' represented >99% reduction from the fruit production of 'Pink Caprice'.

Seed germination. This was conducted as previously reported (Deng et al., 2017). Mature drupes were collected from each plant in the earlier-described replicated field trials in Balm and Ft. Pierce, FL. Seeds were extracted, cleaned, and air-dried at each test site and germinated at IRREC. Due to having few fruit for 'UF-1013-1' and 'Bloomify Red', fruit from four harvests at each site were combined before seed extraction. Seeds were germinated in transparent polystyrene germination boxes (Hoffman Manufacturing, Corvallis, OR) containing two sheets of germination paper (Anchor Paper Company, St. Paul, MN) moistened with 15 mL of water. Germination boxes were placed in temperature and light-controlled chambers equipped with cool-white fluorescent lamps (Model 818; Precision Scientific, Winchester, VA). The germination condition was 12 h light at 25 °C (photosynthetic photon flux was 22 to 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at shelf level) followed by 12 h dark at 15 °C. Germination of seeds was monitored every other day for a period of 60 d. A seed was considered germinated when radicle emergence was ≥2.0 mm. Seeds were removed once germination occurred to prevent inaccurate data collection.

A subsample of seeds of 'Pink Caprice' were also sent to a commercial seed testing laboratory (Midwest Seed Services, Brookings, SD) for initial seed viability tests. The distal end of the cotyledon of each seed was cut off and seeds were stained overnight at 30 °C in 1.0% tetrazolium (2,3,5-triphenyl chloride). Seeds were considered viable if the entire embryo stained evenly. 'UF-1013-1' and 'Bloomify Red' produced few or no seeds at either site and were therefore not subjected to viability tests.

Seeds of 'Pink Caprice' showed an average of 65.0% viability, germinated readily, with an average germination percentage of 45.0% in 60 d (Deng et al., 2017). For 'UF-1013-1', three mature drupes were collected from the Ft. Pierce trial over 4 months. Three seeds were extracted, but all were abnormal when visually examined. Thus, there were no seeds from 'UF-1013-1' for seed viability or germination tests.

FFI. The FFI for 'Pink Caprice' was 4.107 (Deng et al., 2017). Because of the lack of seed germination data, it was not possible to calculate the FFI for 'UF-1013-1'. However, on the basis of its triploidy and extremely low fruit production, it was expected that the FFI for 'UF-1013-1' would be close to 0 and similar to that of 'Bloomify Red'.

Plant Growth, Performance, and Flowering

As previously reported (Deng et al., 2017), lantana plants grown at the two experimental sites were evaluated for plant performance on a scale of 1 to 5, with 1 = few branches, open canopy, lacking vigor; 3 = performing fairly well and acceptably as ornamental plants; and 5 = full plants, dense canopy, desirable shape and color, very attractive. Flower intensity was also rated on a scale of 1 to 5, with 1 = 0% to 20%, 2 = 21% to 40%, 3 = 41% to 60%, 4 = 61% to 80%, and 5 = 81% to 100% of the maximum flower coverage of the plant. At the first 2 months posttransplanting (MPT) and the last evaluation (5 MPT), plant height and width in two directions were measured. On the basis of plant height and width, a plant size index was calculated for each plant in the trials. Data on plant performance, flower intensity, and plant growth were analyzed using the JMP Pro 13.0.2 to determine the significance of differences among cultivars by ANOVA. When differences were significant, mean separation was performed using the Tukey's honestly significant difference procedure.

On average, plants of 'UF-1013-1' grown in Balm were 35.7 cm tall and 51.6 cm wide and had a plant size index of 0.076 m^3 by 2 MPT (Table 4). By 5 MPT, plants of 'UF-1013-1' reached an average height of 47.8 cm and an average width of 86.6 cm (Table 4). Within 3 months (from 2 MPT to 5 MPT), plants increased height by 33.9% and width by 67.8%. Plants of 'UF-1013-1' grown in Ft. Pierce had a similar average height (47.8 cm by 5 MPT), width (76.5 cm) and size index (0.211 m^3) with those grown in Balm (Table 4).

As reported previously (Deng et al., 2017), plants of 'Pink Caprice' were vigorous and large. By 2 MPT, plants were 59.5 cm tall and 103.0 cm wide, and by 5 MPT, they reached an average height of 89.3 cm and an average width of 211.7 cm. By 2 MPT, plants of 'UF-1013-1' were 35.5% to 40.0% shorter in height, 49.9% to 54.4% narrower in width, and 84.5% to 86.6% smaller in size index (Table 4). By 5MPT, plants of 'UF-1013-1' were 46.5% to 50.5% shorter in height, 59.1% to 64.5% narrower in width, and 90.9% to 93.8% smaller in size index than 'Pink Caprice' (Table 4).

Plants of 'UF-1013-1' grown in Balm received 3.0 to 4.7 on plant rating, which were not significantly different from the plant rating scores of 'Bloomify Red' but were significantly higher than the scores of 'Pink Caprice' (Table 5). The flower intensity rating score of 'UF-1013-1' ranged from 1.5 to 3.3, suggesting that 'UF-1013-1' had a lower flower coverage than 'Bloomify Red' in two of the four evaluations and had higher flower coverage than 'Pink Caprice' in three of the four evaluations (Table 5).

Plants of 'UF-1013-1' grown in Ft. Pierce received 2.8 to 3.5 on plant rating and 2.3 to 3.8 on flower intensity rating (Table 5). That is, 'UF-1013-1' and 'Bloomify Red' were not significantly different on plant and flower intensity ratings. 'UF-1013-1' received a lower plant rating score than 'Pink Caprice' in two of the four evaluations and a lower flowering intensity rating in one of the four evaluations (Table 5).

In addition, the plant performance of 'UF-1013-1' was evaluated in three nonreplicated field trials in Citra, FL, from Nov. 2017 to June 2018 (Fig. 3). 'Bloomify Red' was in two of the trials and two to eight commercial lantana cultivars were present in each of the trials. 'UF-1013-1' received the highest scores in two of the trials and a comparable score in the third trial.

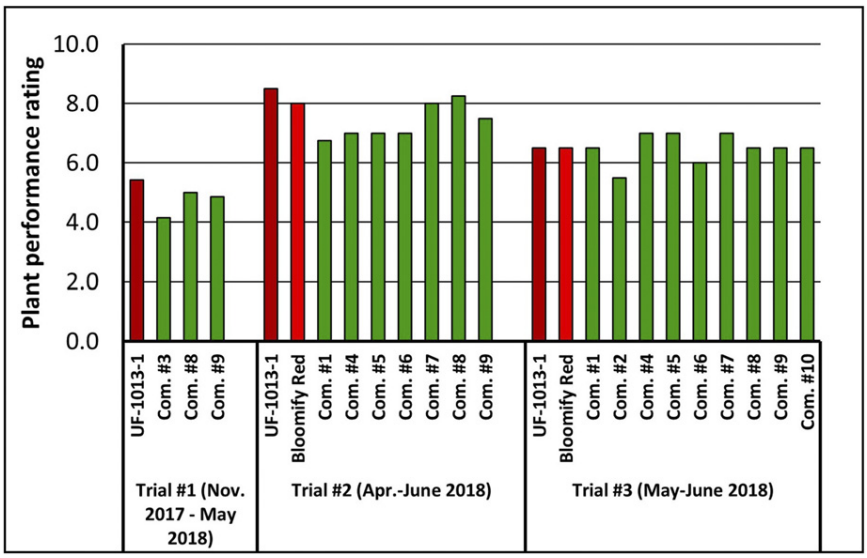

Fig. 3. Overall plant performance ratings of 'UF-1013-1' lantana (*L. camara*) (solid red bars) compared with 'Bloomify Red' (light red bars) and commercial cultivars (Com. 1 to 10) (green bars) in three field trials in Citra, FL, from Nov. 2017 to June 2018. The trials were not replicated but contained five plants per entry in each trial. Ratings were on a scale of 1 to 10, with 1 = poorest, 5 = acceptable, and 10 = excellent. Lantana plants were installed on raised ground beds covered with metalized plastic and grown in full sun.

Hybridization Potential with *L. depressa* after Hand Pollinations

Hand pollination experiments were performed in the greenhouse at GCREC in June and July 2015 to assess the hybridization potential of 'UF-1013-1', as a male or female patent, with *L. depressa*. 'Bloomify Red' and 'Pink Caprice' were included in the hand pollination experiments as sterile and fertile lantana controls, respectively (Deng et al., 2017). Hand pollination was conducted as described by Deng et al. (2017). Plants of *L. camara* cultivars and *L. depressa* were grown on metal benches in gallon plastic containers filled with a commercial soilless mix (Fafard 3B) amended with a controlled release fertilizer (Osmocote, 15N–3.9P–10K, 5- to 6-month release at 21 °C; Scotts) at 7.12 $kg \cdot m^{-3}$. The stock plants were arranged into three blocks, and in each block, they were randomly placed on the benches. The experimental unit was two containerized plants. Temperatures inside the greenhouse ranged from a low of 21 °C at night to a high of 33 °C during day. No supplemental lighting was provided. Plants were drip-irrigated twice a day. Fresh anthers were collected from mature unopened flowers of male parents and applied immediately to emasculated flowers of female parents. At maturity, fruit produced by the pollinated flowers were collected and counted, and seeds were extracted and sown to determine seedling emergence.

As previously described by Deng et al. (2017), 'Pink Caprice', as a male parent, produced an average of 8.6% fruit set on *L. depressa* flowers (Table 6). When pollinated with *L. depressa*, 'Pink Caprice' flowers

Table 6. Hybridization potential of 'UF-1013-1' with *L. depressa* after hand pollinations compared with 'Bloomify Red' (sterile) and 'Pink Caprice' (fertile).

	L. depressa as the female parent			*L. depressa* as the male parent			
Cultivar	Flowers pollinated (no.)	Fruit set (%)	Seedling emergence (%)	Flowers pollinated (no.)	Fruit set (%)	Seedling emergence (%)	References
UF-1013-1	389	0 b[z]	—	496	0 b[z]	—	This study
Bloomify Red	353	0 b	—	558	0 b	—	Deng et al. (2017)
Pink Caprice	388	8.6 a	11.1	452	19.9 a	15.8	Deng et al. (2017)

[z]Fruit set data were arcsine-transformed before analysis of variance was performed in JMP Pro 12.0.1. Means with the same letter within the column are not significantly different by the Tukey's honestly significant difference procedure at $P < 0.05$.

showed 19.9% fruit set (Table 6). Seeds from crosses between 'Pink Caprice' and *L. depressa* or reciprocal showed 11.1% or 15.8% seedling emergence (Table 6). As a male parent, 'Bloomify Red' did not cause any fruit set on *L. depressa* flowers. Nor did it set any fruit after hand pollination with *L. depressa*.

A total of 389 *L. depressa* flowers were pollinated with 'UF-1013-1', and none of the pollinated flowers set fruit, resulting in 0% fruit set (Table 6). When 'UF-1013-1' was used as the female parent, it did not set any fruit after it was hand pollinated with *L. depressa*. Thus, 'UF-1013-1' did not hybridize with *L. depressa* (Table 6). These data confirm the high level of male and female infertility in 'UF-1013-1'.

Conclusion

Plants of 'UF-1013-1' are of moderate vigor, have an excellent branching habit and form a dense, round canopy. Cuttings produce high-quality flowering plants in small and large containers and perform well in the landscape. Flowers of 'UF-1013-1' are yellow/bright red. These attributes make 'UF-1013-1' a desirable replacement of existing fertile lantana cultivars.

Compared with 'Pink Caprice', a *L. camara* cultivar that is the closest to the species' resident taxon (wild or naturalized type), the pollen stainability of 'UF-1013-1' has been reduced by at least 95%. This new triploid cultivar did not cause fruit set on *L. depressa* flowers or set any fruit when hand-pollinated with *L. depressa*. Fruit production of this triploid has been reduced by >99%. Generally 'UF-1013-1' did not produce viable seeds in replicated field trials. The high level of male and female infertility of this triploid was stable from Balm to Ft. Pierce and Citra. These results indicate that 'UF-1013-1' has little potential to hybridize with *L. depressa* and to produce viable interspecific progeny.

Availability

'UF-1013-1' has been licensed to Proven Winners for commercial production and named Luscious® Red Zone™. A plant patent will be applied for this lantana cultivar by the Florida Agricultural Experiment Station. Production of this cultivar will need a licensing agreement with the Florida Foundation Seed Producers, Inc. (http://www.ffsp.net/), P.O. Box 309, Greenwood, FL 32443. Information about plant material and propagation agreement can be obtained from the Florida Foundation Seed Producers, Inc.

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Morphological and Cytological Characterization of Five Porterweed (*Stachytarpheta*) Selections

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***Abstract*. Porterweed (*Stachytarpheta* spp.), a member of the verbena family, is frequently used in pollinator gardens to attract butterflies. This study was conducted to assess the morphological features, pollen stainability and morphology, nuclear DNA content, and chromosome number of five porterweed selections. Coral porterweed (*S. mutabilis*), 'Naples Lilac' porterweed (*S. cayennensis* × *S. mutabilis* 'Violacea'), and nettleleaf porterweed (*S. cayennensis*) had the largest plant heights. Flower number was significantly higher in nettleleaf porterweed, jamaican porterweed (*S. jamaicensis*), and U*J3-2 porterweed (*S. cayennensis* × *S. jamaicensis*), with an average of 65–72 flowers per inflorescence. Internode length and flower width of jamaican porterweed had much lower values than the other selections. Coral porterweed recorded the lowest pollen stainability with only 10.6% stainability, but it had the largest relative pollen production. 'Naples Lilac' porterweed had the highest DNA content with an average of 3.79 pg/2C, like jamaican porterweed with 3.73 pg/2C. Ploidy levels varied between selections, and the basic chromosome number was $x = 28$. Coral, jamaican, and 'Naples Lilac' porterweed had $2n = 6x = 168$ chromosomes, first reported in this genus. These results provide a guide and a new tool to distinguish native and non-native porterweed and may aid future breeding toward the production of noninvasive cultivars.**

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Porterweed (*Stachytarpheta* spp.) attracts numerous butterfly species and other pollinators and is often planted in pollinator gardens (Gilman, 2014). It is a low-maintenance plant with high drought tolerance and is commonly used in the southern United States in cold hardiness zones 9b through 11 (Lamborn, 2017). Porterweed produces flowers from summer through fall before dying back during winter. The *Stachytarpheta* genus is vast, with 133 species identified in Australia (Munir, 1992) and 79 species classified in Brazil (Atkins, 2005). Seed is readily available to the public and can be found from many nurseries and online sellers. In the wild, seed is spread through the wind, vehicles, and machinery (The State of Queensland, 2016).

In Florida, the most sold porterweed species are jamaican porterweed (*Stachytarpheta jamaicensis*), nettleleaf porterweed (*Stachytarpheta cayennensis*), coral porterweed (*Stachytarpheta mutabilis*), purple porterweed (*Stachytarpheta frantzii*), and dwarf red porterweed (*Stachytarpheta sanguinea*) (http://plantANT.com). Jamaican porterweed is native to dunes, shell middens, pine rocklands, and disturbed sites of central and southern Florida (Wunderlin and Hansen, 2011); whereas nettleleaf porterweed was introduced to the United States from Central and South America and has escaped cultivation (USDA, NRCS, 2020). While it has not yet altered native plant communities in Florida, nettleleaf porterweed is listed as a Category II invasive plant by the Florida Exotic Plant Council (FLEPPC) due to its increased abundance or frequency (FLEPPC, 2019). The University of Florida Institute of Food and Agricultural Sciences's (UF/IFAS) status assessment of nonnative plants in Florida's natural areas is to "use with caution" (UF/IFAS Assessment, 2020). Hybridization potential between the native and invasive congeners is of concern. For example, in Florida, the introduced lantana (*Lantana camara*) has hybridized with the native pineland lantana (*Lantana depressa*), contaminating the gene pool of the native species (Hammer, 2004; Sanders, 1987). Proper identification and distinction between native and invasive species are crucial components of reducing the spread of invasive ornamentals (Steppe et al., 2019). Although plant experts could observe some differences between jamaican and nettleleaf porterweed (Wunderlin and Hansen, 2011), these two species share a lot of similarities in plant morphology. These similarities make it difficult to identify them reliably before making decisions for invasive plant management, especially for those who are not familiar with porterweed plants.

In prior work, Wilson et al. (2009) evaluated seed production and viability of eight porterweed selections in Florida and found three of them to be highly female sterile. Female sterile porterweed included the following: 'Violacea' porterweed (*Stachytarpheta mutabilis*), 'Naples Lilac' porterweed (*S. cayennensis* × *S. mutabilis* 'Violacea'), and 'Mario Pollsa' porterweed (*Stachytarpheta* spp.). Through controlled crosses, the potential for nettleleaf porterweed to hybridize with jamaican porterweed was realized. However, there is a lack of information, such as ploidy level and pollen stainability on these porterweeds, which would be useful data in the breeding of noninvasive plants. Pollen staining has become a reliable method of determining pollen viability in hybridization studies (Czarnecki et al., 2014). Other porterweed studies have described pollen morphology and stainability for *S. angustifolia*, *S. cayennensis*, *S. chamissonis, S. indica*, and *S. sericea* (Adedeji, 2010; Atkins, 1991; Solanke et al., 2019). These studies revealed diverse morphologies and stainability of pollen grains.

Chromosome number and ploidy level are important plant characteristics; the latter is an

important factor in determining plant fertility and hybridization potential. It was previously reported that the porterweed genus has varying numbers of chromosomes, from $2n = 18$ to $2n = 160$, and varying levels of ploidy (Fedorov, 1974; Sanders, 2001). Flow cytometry has become a widely used tool to determine nuclear DNA content and ploidy level of plants due to high sample throughput (Doležel et al., 2007). Polyploids have been identified in porterweed using flow cytometry, but exact ploidy levels need to be determined or confirmed by chromosome counting (Wilson et al., 2009). To accurately interpret the ploidy level based on nuclear DNA content, somatic chromosomes must be determined to provide references for comparing nuclear DNA content (Doležel et al., 2007).

The purpose of this study was to characterize morphological and cytological features of five representative porterweed selections. The main objective was to count their chromosomes in root tip cells, determine nuclear DNA contents by flow cytometrical analysis of leaf tissues, and understand the relationship between ploidy level and nuclear DNA content in porterweed. A secondary objective was to assess pollen morphology and stainability among these porterweed selections. This information, along with complete leaf and flower morphological profiling, will improve identification, promote the use of native porterweed, and control the further spread of invasive porterweed.

Materials and Methods

Plant materials. Five porterweed selections were evaluated in this study. Coral, jamaican, 'Naples Lilac', and nettleleaf porterweed plants were obtained from a previous study conducted by Wilson et al. (2009). U*J3-2 resulted from controlled crossing of *S. cayennensis* and *S. jamaicensis* in a greenhouse located at the UF/IFAS Gulf Coast Research and Education Center (Wimauma, FL). Vegetatively propagated porterweed plants were grown in 3.8-L plastic containers filled with Fafard 2P mix (Florida Potting Soil, Orlando, FL). Container-grown plants were hand-watered daily and fertilized with a controlled-release fertilizer (Osmocote; 15N–3.9P–10K, 5 to 6 months release at 21 °C; The Scotts Company, Marysville, OH) at 6.51 $kg \cdot m^{-3}$. Plants were grown between July and Nov. 2014 under natural light in a temperature-controlled greenhouse with a temperature of 29.4 °C during the day and 21.1 °C at night.

Morphological characterization. Mature flowering plants (≈100 d after cuttings were made) were used to collect plant morphological data. Growth habit was identified as upright, semiupright, or prostrate. Plant height, plant width for two perpendicular axes, and inflorescence length were recorded using a standard wooden meter stick. Leaf length, leaf width, and internode length were measured using a stainless-steel ruler. Internode diameter and flower width were determined using an electronic digital caliper (Fowler & NSK Max-Cal, Japan). Five replicates (five individual plants) were accessed for each porterweed selection.

Determining nuclear DNA content. An Accuri C6 flow cytometer (BD Biosciences, San Jose, CA) at the UF's Interdisciplinary Center for Biotechnology Research (ICBR, Gainesville, FL) was used to determine nuclear DNA content. The flow cytometry protocol recommended by Doležel et al. (2007) was followed using rye [*Secale cereal* 'Daňkovské' (16.19 pg/2C)] as the internal standard. One mL of the LBO1 lysis buffer (Doležel et al., 2007) was added to a petri dish, and ≈30 mg of tender leaf tissue of porterweed and rye were chopped together using a razor blade to release nuclei. The nuclei preparation was filtered through a nylon mesh (50 µm) into a loading tube, and then 50 µL of the DNA fluorochrome propidium iodide (Sigma-Aldrich; 1 $mg \cdot mL^{-1}$) and RNase (Sigma-Aldrich; 1 $mg \cdot mL^{-1}$) were added. The nuclei-containing solution was fed into the flow cytometer. Three flow cytometrical analyses were run for each porterweed selection, and a minimum of 3000 nuclei were counted per run. Nuclear DNA content (pg/2C) was calculated according to Doležel et al. (2007): sample DNA content = nuclear DNA content of internal standard ('Daňkovské' rye) × (mean fluorescence value of sample ÷ mean fluorescence value of internal standard).

Squashing and counting chromosomes. The cell wall degradation hypotonic method of Chen et al. (1982) was used. Before 10:00 AM, vigorously growing root tips (1 cm) were excised from porterweed plants and treated in 0.002 M 8-hydroxyquinoline for 4 h in the dark. Root tips were fixed in a fixative solution (3 methanol: 1 acetic acid, v/v) for at least 2 h. The fixed roots were rinsed three times in deionized water before a much smaller section of the root tips (≈1 mm) was excised and macerated in an enzyme solution containing 2.5% cellulase and 2.5% pectinase for 3 h 15 min inside an incubator at 27 °C. After incubation, macerated root tips were washed in deionized water for 10 min and then fixed in a fixative solution (3 methanol: 1 acetic acid, v/v) for 0.5 h. Root tips were squashed in a drop of the fixative solution on a pre-chilled microscopic glass slide. The prepared slide was heated over an alcohol burner for a few seconds and stained with a 2.5% Giemsa solution for 10 min. Stained glass slides were rinsed in distilled water, air-dried, and then observed under a BX41 microscope with an Olympus Q-color 5 camera (Olympus America Inc., Melville, NY). Darkly stained and well-spread chromosomes were photographed at ×1000 magnification.

Examining and staining pollen grains. Flowers were collected just before opening and dissected to reveal anther sacs. Predehiscent anthers were removed and immersed in 100 µL of cotton blue stain in microcentrifuge tubes. Tubes were placed in a 65 °C water bath where anthers were stained overnight. Stained anthers were rinsed three times in water, squashed in 50 µl of 80% glycerol on a microscopic glass slide, and then covered with a glass coverslip. A BX41 microscope with an Olympus Q-color 5 camera (Olympus America Inc.) was used to observe pollen grains at ×40 and ×100 magnification. Images of pollen grains were taken and analyzed to calculate pollen stainability. Pollen grains stained dark blue were considered stainable. Measurements of pollen grains were taken using ImageJ 1.52s (U.S. National Institutes of Health, Bethesda, MD). At least 12 anthers from three inflorescences were analyzed for each porterweed selection. Two slides were prepared for each selection, and three fields of view were selected at random and photographed. Each pollen grain was counted and used to calculate relative pollen stainability. Pollen grain density was compared with other selections for relative production.

Statistical analysis. Analysis of variance was performed in JMP Pro 15.0.0 (SAS Institute, Cary, NC) to determine if there were significant differences among porterweed selections in plant height and width, leaf length and width, inflorescence number, flower number, flower width, and pollen stainability. When differences were significant at $P \leq 0.05$, mean separation analysis was performed in JMP Pro 15.0.0 using the Tukey–Kramer honestly significant difference procedure at $P \leq 0.05$. Linear regression was performed using JMP Pro 15.0.0 to determine if a correlation existed between two traits. The Pearson correlation test was used to calculate the correlation coefficient.

Results and Discussion

Morphological characterization. Remarkable variability was observed among the morphological features of the five porterweed selections. Porterweed growth habits were categorized as upright, semiupright, and prostrate, with two selections each falling into upright and prostrate, and nettleleaf identified as semiupright (Table 1, Fig. 1). Coral and 'Naples Lilac' plants were upright with shoots extending upwards. Coral porterweed showed little branching with pink-to-coral flowers, while 'Naples Lilac' had a fuller appearance with purple flowers (Fig. 2A). Nettleleaf porterweed exhibited a semiupright growth habit with darker-purple flowers. Plants of this selection grew partially to the side of the pot, extending away from the base and partially extending upward. U*J3-2 and jamaican porterweed were both prostrate with low, spreading growth. Both selections had high branching, but jamaican porterweed filled the area surrounding all sides of the pot instead of just one side. U*J3-2 displayed purple flowers and jamaican porterweed had light purple. Leaf morphology was identified as ovate for all selections (Fig. 2B). Classification of these growth habits and flower colors alone provides substantial clues to identifying these porterweed selections. For example, distinguishing invasive nettleleaf from the native

Table 1. Plant, leaf, and flower morphological data of five porterweed selections. Data were collected on five plants of each selection grown at the University of Florida's Gulf Coast Research and Education Center.

	Taxa	Growth habit	Plant ht[z]	Plant width	Leaf length	Leaf width	Internode length	Internode diam	Inflorescence length	Flower number	Flower width
Coral	(*S. mutabilis*)	Upright	60.1 a	61.2 b	23.1 a	10.3 a	9.8 ab	12.2 a	62.8 a	11.2 c	1.7 a
Naples Lilac	(*S. cayennensis* × *S. mutabilis* 'Violacea')	Upright	59.8 a	63.9 b	15.6 b	7.2 b	9.8 ab	13.1 a	66.1 a	30.2 b	1.5 b
Nettleleaf	(*S. cayennensis*)	Semi-upright	46.0 b	62.3 b	13.7 b	6.2 bc	10.1 a	12.4 a	45.4 b	71.6 a	1.3 c
U*J3-2	(*S. cayennensis* × *S. jamaicensis*)	Prostrate	36.1 b	80.0 a	14.7 b	6.4 b	11.8 a	12.3 a	68.8 a	65.8 a	1.3 c
Jamaican	(*S. jamaicensis*)	Prostrate	18.1 c	63.2 b	12.9 b	5.0 c	7.1 b	7.9 b	67.8 a	65.0 a	1.0 d

[z]Means followed by a common letter within each column are not significantly different by Tukey's honestly significant difference test at the 5% level of significance.

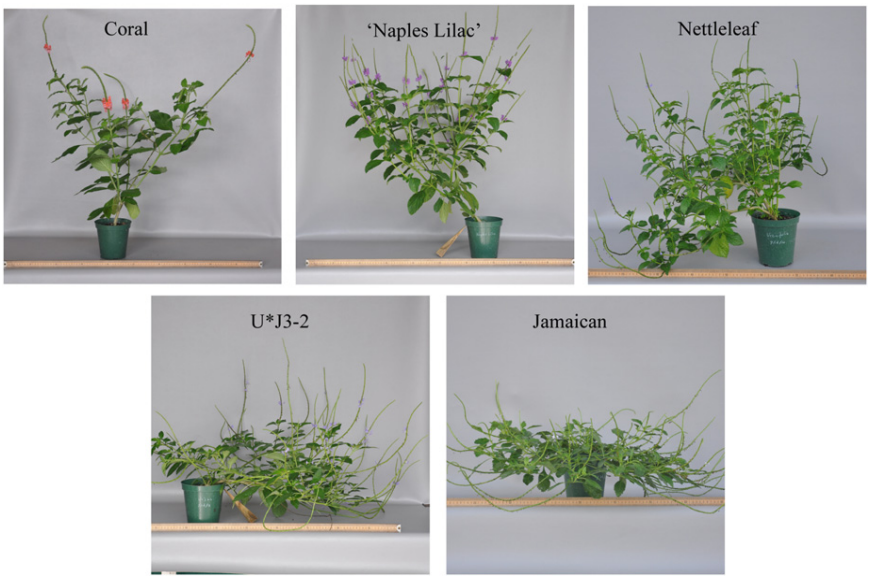

Fig. 1. Images of each porterweed selection at the time of data collection. Plants were propagated at the same time and grown under the same conditions.

jamaican porterweed can be determined by assessing the direction of growth and the intensity of purple colors in the flowers.

The range of flower number per inflorescence varied greatly, with a maximum of 80 flowers in nettleleaf porterweed and a minimum of 8 flowers per inflorescence in coral porterweed. Flower number was fewer in coral porterweed, with an average of only 11.2 flowers. Plant height was another morphological feature that largely differed between selections, ranging from 60.1 cm in coral porterweed to 18.1 cm in jamaican porterweed. Coral porterweed had larger leaves than the other selections, with an average length of 23.1 cm and a width of 10.3 cm (Table 1). U*J3-2, a line resulting from an interspecific cross between nettleleaf and jamaican porterweed, showed a significantly higher plant width of 80 cm, compared with both parents and all other selections in the study.

Recording few numbers of flowers per inflorescence, coral porterweed had the highest average flower width of 1.7 cm. 'Naples Lilac' had a high plant height of 59.8 cm and the highest average internode diameter of 13.1 mm. Nettleleaf porterweed had a relatively small inflorescence length of only 45.40 cm (Fig. 2C). Inflorescence length was the highest in U*J3-2, extending 68.8 cm. Jamaican porterweed was the shortest plant in the study, only reaching 18.1 cm in height. It had the smallest leaf width of 5.0 cm and a small flower width of only 1 cm. It is of interest to note that U*J3-2 was similar in plant height, internode length, internode diameter, and flower width to its female parent (nettleleaf porterweed), but it was similar in leaf length, leaf width, and flower number to both of its parents. The hybrid's plant width was significantly wider than both parents. Similar segregation of traits was observed in the hybridization of *S. angustifolia* × *S. cayennensis* produced by Solanke et al. (2019).

These results were consistent with prior findings (Wilson et al., 2009) where flower quality was assessed monthly for 7 months and rated 1–5 based on the number of spikes and number of open flowers. Coral porterweed had fewer flower spikes and lower flower-quality ratings than other selections, while nettleleaf porterweed had the most flower spikes resulting in overall higher flower ratings. While flower quantity can be important for both aesthetic appeal and attraction of pollinators, a negative consequence in the case of an escaped non-native ornamental is seed production and subsequent seedling establishment. In the prior study, it was estimated that an average coral porterweed plant has the potential to produce only 70 seedlings per growing season, whereas nettleleaf porterweed has the potential to produce 26,600 seedlings.

Identifying porterweed by flower color alone can be difficult without thorough experience working with the genus. The morphological traits identified in this study such as leaf size and growth habit may enable us to identify porterweed in ecosystems with greater accuracy. Also, the growth habit and inflorescence length of the invasive nettleleaf porterweed are important features that can aid in identifying and removing this species in the United States.

Nuclear DNA content. Standard deviation values for the mean nuclear DNA content of five porterweed selections were ≤0.09 pg, suggesting that this method is robust (Table 2). Nuclear DNA content for the selections ranged from 2.81 pg/2C in nettleleaf porterweed to 3.79 pg/2C in 'Naples Lilac'. This is of interest, because 'Naples Lilac' is reported to be a cross between the invasive nettleleaf porterweed and the non-invasive 'Violacea' porterweed (*S. mutabilis*) (Kastenholz, personal communication). Coral and jamaican porterweed had similar DNA contents of 3.66 pg/2C and 3.73 pg/2C. The nuclear DNA content of U*J3-2 was 3.28 pg/2C, close to the average of the nuclear DNA content of jamaican and nettleleaf porterweed. Nuclear DNA content data for the genus *Stachytarpheta* are not available in the plant DNA C-value database (Pellicer and Leitch, 2019). Our data will help fill this gap in the widely used database.

Chromosome counts. A total of 182 well-spread metaphases were observed, photographed, and counted to determine the somatic chromosome number for all five selections (Fig. 3). At least 31 metaphases were observed for each selection, which revealed three chromosome numbers ($2n = 4x = 112$, $2n = 5x = 140$, and $2n = 6x = 168$) (Table 2). Coral, jamaican, and 'Naples Lilac' porterweed recorded a large somatic chromosome number of 168. U*J3-2 porterweed had 140 chromosomes, and nettleleaf

Fig. 2. Morphology of porterweed open flowers (**A**), leaves (**B**), internodes (**C**), and inflorescences (**D**). Left to right: Coral porterweed, 'Naples Lilac' porterweed, nettleleaf porterweed, U*J3-2 porterweed, and jamaican porterweed.

Table 2. Nuclear DNA content and chromosome number of five porterweed selections. Three flow cytometrical analyses were run for each porterweed selection.

Taxa	Nuclear DNA content ± SD (pg/2C)	Metaphases observed (no.)	Chromosome number	Base chromosome number	Ploidy level
Coral	3.66 ± 0.05	38	168	28	6*x*
Naples Lilac	3.79 ± 0.04	41	168	28	6*x*
Nettleleaf	2.81 ± 0.03	36	112	28	4*x*
U*J3-2	3.28 ± 0.05	36	140	28	5*x*
Jamaican	3.73 ± 0.09	31	168	28	6*x*

porterweed had 112 somatic chromosomes. All selections have the same basic chromosome number ($x = 28$).

Literature indicated $2n$ = 18, 48, 56, ≈120, and 160 chromosomes in *Stachytarpheta* (Sanders, 2001). These chromosome numbers were based mostly on observations in *S. cayennensis* (Sanders, 2001), and there has been a lack of information on the chromosome numbers for many other *Stachytarpheta* species (Rice et al., 2015; Sanders, 2001). Our chromosome counting data represent the first for *S. jamaicensis* and *S. mutabilis* and the largest chromosome numbers in the genus. This is also the first report of a chromosome number for a hybrid between nettleleaf and jamaican porterweed, which was identified as a pentaploid.

There was a linear positive relationship ($R^2 = 0.9831$, $P < 0.001$) between chromosome number and DNA content in these selections. This positive relationship may allow inferences of ploidy level to be made based on nuclear DNA content in these species.

Effects of polyploids on plant, stem, leaf, and flower morphology have been documented in many plants. The existence of three ploidy levels among these selections prompted us to perform regression analysis to find out if there were any relationships between ploidy level and morphological parameters [including plant height and width, leaf length and width, internode length and diameter, flower number (per inflorescence) and width, and length of inflorescences]. A strong linear relationship was observed between ploidy level and length of inflorescences ($R^2 = 0.7970$, $P < 0.0001$), and plant width ($R^2 = 0.7092$, $P < 0.001$) (Table 3). A similar relationship was also observed between ploidy level and flower number ($R^2 = 0.4477$, $P < 0.0015$) and internode length ($R^2 = 0.3175$, $P < 0.0150$). Such a relationship was not detected with plant height, leaf length and width, internode diameter, or flower width. It should be pointed out that because of the limited number of selections available for this study, these linear relationships should be considered preliminary. Many more selections of different ploidy levels should be assessed to gain a better understanding of the effects of polyploidy on porterweed morphology.

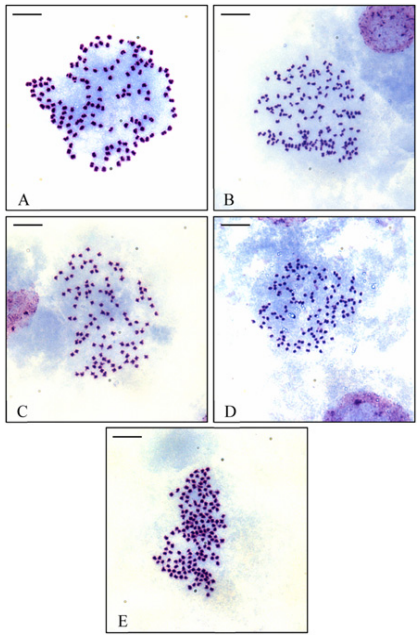

Fig. 3. Micrographs (×1000) of somatic chromosomes observed in root tip cells stained in Giemsa. (**A**) Coral porterweed ($2n$ = 168). (**B**) 'Naples Lilac' porterweed ($2n$ = 168). (**C**) Nettleleaf porterweed ($2n$ = 112). (**D**) U*J3-2 porterweed ($2n$ = 140). (**E**) Jamaican porterweed ($2n$ = 168). Scale bar = 10 µm.

The identification of polyploids is necessary for breeders to use ploidy manipulation

Table 3. Summary results from regression analysis to detect potential relationship between ploidy level and porterweed plant, leaf, stem, flower, and inflorescence morphological parameters.

	Plant		Leaves		Internodes		Flowers		
	Ht	Width	Length	Width	Length	Diam	Number	Width	Inflorescence length
R^2	0.0554	0.7092	0.1389	0.0968	0.3175	0.0987	0.4477	0.0390	0.7970
P	0.5345	<0.0001	0.1931	0.3265	0.0150	0.3190	0.0015	0.6454	<0.0001

Table 4. Relative quantity, shape, size, and stainability of pollen grains of five porterweed selections. At least twelve anthers from three inflorescences per selection were used for analysis.

Taxa	Relative pollen production	Shape	Pollen length Pollen ± SD (μm)[z]	stainability Pollen ± SD (%)[z]
Coral	++++	Triangular	252.1 ± 9.8 a	10.6 ± 3.6
Naples Lilac	+	Round	143.6 ± 9.5 c	—
Nettleleaf	++	Triangular	174.3 ± 9.2 b	44.0 ± 8.1
U*J3-2	+	Variable (round, irregular)	130.5 ± 5.0 c	—
Jamaican	++	Triangular	180.3 ± 3.2 b	51.4 ± 6.4

[z]Means followed by a common letter within column are not significantly different by Tukey's honestly significant difference test at the 5% level of significance. "—" indicates further studies needed.

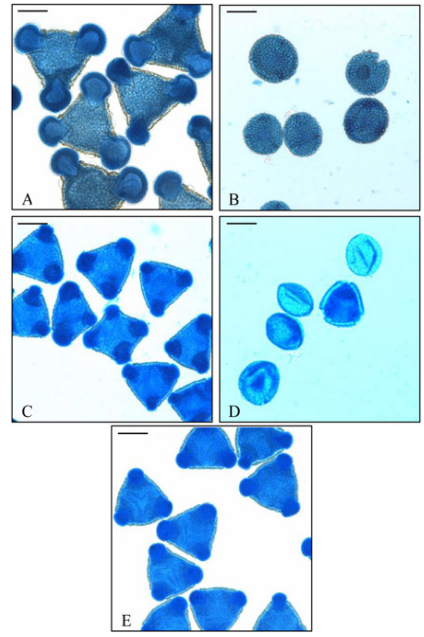

Fig. 4. Micrographs (×100) of pollen grains stained in cotton blue for 5 h. (**A**) Coral porterweed. (**B**) 'Naples Lilac' porterweed. (**C**) Nettleleaf porterweed. (**D**) U*J3-2 porterweed. (**E**) Jamaican porterweed. Scale bar = 100 μm.

in generating noninvasive sterile cultivars. Sterile cultivars of *Lantana camara* have successfully been developed by the crossing of tetraploids and diploids (Czarnecki et al., 2014; Deng et al., 2017, 2020). U*J3-2 was identified as a pentaploid in this study and will need further research to assess its degree of male and female sterility or infertility. Wilson et al. (2009) identified 'J.P.'s Pink' (*S. speciosa*) and 'Red Compact' (*S. speciosa*) porterweed as diploid species, making them potential candidates for crosses with now-identified tetraploid nettleleaf porterweed. Furthermore, confirming polyploids through chromosome staining has laid the groundwork for determining the ploidy of other porterweed species with flow cytometry.

Quantity, morphology, and stainability of pollen grains. Pollen production varied between selections, with coral porterweed having the largest relative pollen production (Table 4). Pollen staining was reported only for coral, jamaican, and nettleleaf porterweed. Reliable staining results were not available for 'Naples Lilac' and U*J3-2, two interspecific hybrids. Future research is needed using different staining procedures or pollen germination to determine their pollen stainability or viability.

Coral, jamaican, and nettleleaf porterweed had triangular pollen grains, as opposed to 'Naples Lilac', which had round pollen grains; and U*J3-2 had variably shaped pollen grains (Fig. 4). Variability was observed in the size of pollen grains among these selections. Coral porterweed had the largest pollen grains, with an average length of 252 μm. Jamaican and nettleleaf porterweed had much smaller pollen, measuring 180.3 μm and 174.3 μm, respectively. 'Naples Lilac' and U*J3-2 had diameters of 130.5–143.6 μm. Average pollen diameter for nettleleaf porterweed was reported as 116.5 μm by Adedeji (2010), differing from this study by 57.8 μm. However, the previous study observed high variability in pollen grain size and observed several different pollen shapes, perhaps accounting for the difference in diameter. No linear relationship was detected between ploidy level and pollen grain size in these porterweed selections when the pentaploid U*J3-2 was included or excluded (data not shown).

Coral porterweed recorded the lowest pollen stainability, with only 10.6% stainability. Nettleleaf and jamaican porterweed had 44.0% or 51.4% pollen stainability, and they also had the highest fruit set and seed viability in the previous study (2009). High male and female fertility have contributed to the presence of these two selections in the southern ecosystems. Solanke et al. (2019) reported pollen stainability for nettleleaf porterweed at 87.8%, nearly double that observed in the present study. This difference could be due to the duration of cotton blue staining, where the former study allowed pollen to stain for 48 h. Pollen stainability results for 'Naples Lilac' and U*J3-2 were not presented in this study due to issues with staining of the pollen grains of these two selections.

Pollen staining results indicate that coral porterweed is the only selection analyzed with potential male sterility. This finding, along with lower female fertility identified in the previous study, makes coral porterweed a possible infertile alternative for landscapes. More research is needed to understand why coral porterweed was not like the other two hexaploids (jamaican and 'Naples Lilac') but showed a much lower pollen stainability.

In summary, species evaluated within the porterweed complex differed widely in their leaf and floral morphology, chromosome number, and pollen stainability. This is a first report confirming ploidy level variation among these selections. Our study showed that the Florida native (jamaican porterweed), the introduced species (nettleleaf porterweed), and their progeny (U*J3-2) are distinctly different in nuclear DNA content and chromosome number. These differences should provide a reliable tool to differentiate the native from the introduced porterweed in cases where morphological identification becomes difficult. This information may not only be useful in future breeding programs, but also may be a consideration for selecting noninvasive traits. Of the porterweed selections examined, coral porterweed stands out as having low pollen stainability and low female fertility that were reduced by 76% and 83%, respectively, compared with the invasive nettleleaf porterweed. Comprehensive morphological comparisons among porterweed selections presented herein will greatly aid in their proper identification and sustainable use in Florida.

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Connecting with Small Scale, Urban, Hobby, and Community Garden Farmers through Video Mediums and Facebook Live

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Small scale producers often visit non-science-based websites in search of general and specific information about horticulture and livestock, disease and pest pressure and cultural conditions required to grow crops. During the COVID-19 pandemic, alternative educational outreach was required to continue supporting the needs of agricultural producers. Several Sustainable Agriculture and Food Systems Extension Agents teamed up and utilized Facebook Live or posted videos on their county Facebook page to reach wide range of urban and rural small-farm producers on an array of agricultural topics. Using this platform gives exposure to science-based information on social media platforms where many producers are already active. An added benefit to using Facebook Live and videos is that community gardeners and home gardeners can access the same information, which provides them with tools for increased success in growing food for home consumption. From zero downloads in March 2020, the downloads grew to more than 6000 views by August 2020. As of August 2020, 21% (n = 1207) of the viewers engaged in comments and 12% (n = 146) the viewers who commented requested additional information. Many growers have expressed their appreciation for the information in this alternative teaching style.

Changing workloads and demographics have compelled extension professionals to move from face-to-face to virtual teaching over the past decade. The coronavirus pandemic forced professionals across the world to increase guidance, education, and facilitation of peer-to-peer interactions online. The benefit of this shift is reaching new audiences and finding innovative ways to meet the land-grant extension mission of increasing knowledge, changing behavior, and assessing the impacts (Gharis et. al, 2014).

Small-scale producers often use social media for advertising, marketing their farm products, and to educate consumers about how food is grown and where it comes from. Platforms that producers utilize most often include Facebook, YouTube, blogs (Gharis et. al, 2014) and more recently Instagram. Small farmers may not necessarily reach out to extension services to find information regarding horticulture, livestock, disease and pests, cultural conditions, or information about programs available to help sustain their operations such as food safety or business planning. Finding the University of Florida extension science-based resources broadcasted on social media platforms where they may already be active, can be a bonus for small farm producers. In addition to small-scale producers, hobbyist, urban and community gardeners, entrepreneurs, and consumers can access the same information, which can provide tools for increased success in growing food for home consumption and increased knowledge about food production.

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Additionally, Pew Research Center data roughly estimates 69% of adults pursue social media for socializing, catching up on the news, and as a learning platform (Anon. 2020a) Consumers who visit non-peer reviewed resources may receive inaccurate or misleading information about how food is grown, harvested, processed, and distributed. Farmers have been leaders in correcting misinformation regarding food production to the average consumer, often using social media. The spread of misinformation on social media platforms has been heavily documented across sectors including agriculture (Jiang and Wang, 2019). However, research also shows that the public is more likely to say they regularly see content that introduces them to a new idea than to say they regularly see content that seems obviously false or untrue or that provokes negative feelings toward others (Anon. 2020b).

Materials and Methods

Several Sustainable Agriculture and Food Systems extension agents teamed up to utilize informal videos on social media platforms such as Facebook Live, YouTube, Instagram, and blog posts on their county webpages to reach a wide range of urban and rural small-farm producers on an array of agricultural topics such as specialty vegetable crops, beekeeping, livestock management, tropical fruit and vegetable production, small poultry flocks, on-farm readiness reviews, farm food safety and more. Agents engaged community partners and work colleagues to enhance the diversity of speakers and expert topics. Agents also linked and shared content between county social media platforms to get a wider impact with the audience.

Results and Discussion

From zero downloads in March 2020, downloads as of August 2020 grew to more than 9,453 views, with 38% (n=3,646) of the viewers engaged in posts and 5% (n=173) of the engaged viewers following up with the Agents to request additional information.

- Reached = 33,051.
- Views = 9453.
- Engaged (likes, shares, comments) = 3646.
- An average of 82% of the viewers were from Florida but sometimes as far away as Alaska and Europe.
- Increase in viewership as increased video availability.
- Producers and gardeners were using science-based information.

Extension's mission is to take timely, practical, and science-based resources to stakeholders. In addition, the added convenience of plugging into platforms where many people are already actively looking for reliable information during uncertain times allowed for several benefits and challenges. Since producers are already active on social media for marketing purposes, agents attempting to meet them on these platforms not only expanded the mission of extension but the increased the likelihood that producers would utilize science-based information.

Benefits included an increase in clientele reached in a short period; immediate response to stakeholder questions during live sessions, general ease of outreach efforts without high video production, and the ability to make the information ADA accessible. In addition, agents developed new skills for future programming. Extension professionals are required to track impact of their programs, making social media a useful tool that automatically tracks interactions, views, comments, and conversations. Additionally, consumers, community gardeners and home gardeners can access the same information as small farm operators, thereby providing them tools for increased success in growing food for home consumption and an increase knowledge about how food is produced.

A significant challenge was the learning curve utilizing Zoom, the different social media platforms, and the equipment criteria. As familiarization of the technology increased, glitches in technology occurred such as a Facebook hack, overheated phones, distorted pictures, subpar microphone quality on devices, social distancing causing a variant in speaker's volume, and low storage capabilities on mobile devices. Each county has different standards for video formats and accessibility. There was a learning curve to find the best time for posting information; some videos had too few viewers while other videos had so many comments the presenter could not respond during the Facebook Live session.

An important note is that a significant number of producers in the four counties studied do not have internet access. In some parts of the state of Florida, as many as one out of every three producers do not having internet access.

- 73% of DeSoto County producers have internet access.
- 76% of Manatee County producers have internet access.
- 77% of Sarasota County producers have internet access.
- 84% of Collier County producers have internet access.

(USDA, 2017a, USDA 2017b, USDA 2017c, and USDA 2017d)

Conclusion

While online videos may not bet readily accessible to approximately 25% of the targeted audience, the benefits of using social media platforms outweigh the challenges and inaccessibility. The producers without internet access continue to utilize phone calls and emails to gather science-based resources from the University of Florida Extension Sustainable Agriculture and Food System Agents during COVID-19.

The need for extension agents to interact with stakeholders in person and participate in hands-on teaching is still an important tool for building trust and relationships within the agricultural community. However, the shift to reliable online information in an urgent time proved to be a benefit to stakeholders.

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Growth of Arugula In a Hydroponic Ebb and Flood System Supplemented with *Lactobacillus*

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This study investigated whether the addition of *Lactobacillus casei* to a hydroponic system would increase the yield of arugula (*Eruca vesicaria* ssp. *sativa*) when compared to the control. Arugula was grown using an ebb and flood system with 0, 7, 14, or 21 oz of EM-1 (*L. casei*) added to the recirculating nutrient solution. Shoot and root growth, nutrient levels in the hydroponic solution and the leaves, and colony-forming units in solution and on the roots were measured. The addition of *L. casei* did not improve yield, and plant growth was similar among treatments. There was also no difference in nutrient levels in the hydroponic solution among the treatments, suggesting that *L. casei* did not improve nutrient uptake. This finding was supported by minimal differences in tissue nutrient levels. The number of CFU's in the nutrient solution decreased each week from levels measured at the beginning of the week. The lowest number of CFUs in solution were for control plants. The CFU recorded on roots was greatest on plants grown with the 21-oz treatment. It appears that *L. casei* was living on the roots and in the solution. Improved growth with the addition of microbes is often observed when plants are exposed to abiotic or biotic stress. However, when plants are grown under optimum conditions, the benefits of adding microbes are often undetectable.

Arugula (*Eruca vesicaria* ssp. s*ativa*) is an annual in the Brassicaceae family. Many of the plants in this family are popular because of the diverse populations of phytochemicals in their leaves. For example, sulforaphane and erucin found in arugula leaves have been associated with positive health benefits (Wassermann et al., 2017). Arugula also is very high in glucosinolate, which are suggested to play a role against carcinogenesis, metastasis and protect cells from chronic inflammatory diseases (Wassermann et al., 2017). With the growing demand for food with high nutrient content, arugula has become more popular and has a large following in the health food stores because of its beneficial properties. Arugula is widely sold as a specialized herb and it is commonly found in salad mixes.

Due to arugula's limited growing season but year-round demand, hydroponic growers are able to fill the gap in production. The versatility of hydroponics makes it an attractive form to grow a variety of crops in a multitude of ways. Like lettuce (*Lactuca sativa*), arugula grows well in hydroponic systems that are aerated and have adequate spacing between plants to limit diseases (Lee and Jiyuang, 2015). Commercial growers have also started experimenting with adding beneficial microorganisms to hydroponic systems in order to improve plant growth and protect plants from diseases.

Most rhizospheres can contain up to 100 million to 1 trillion microorganisms in a single gram (Kennedy and de Luna, 2005). However, the hydroponic rhizosphere is suspected to be less diverse than soil, so the effects of adding microorganisms into the nutrient solution might have an impact on plant growth. Hydroponics is limited in organic nutrients that could potentially give growers more fertilizer options. By studying microorganisms that can survive in a hydroponic system, we might help growers protect their plants from disease in addition to improving nutrient absorption. The objective of this study was to determine if the addition of *Lactobacillus casei* to a hydroponic system would increase the yield of arugula when compared to the control. *L.casei* is widely known to have a wide range of pH and temperature in which they can thrive and is considered generally safe (GRAS) for use in a hydroponic study (De Angelis and Gobbetti, 2011).

Materials and Methods

Arugula seeds (Johnny's Selected Seeds, Winslow, ME) were sown directly into 3.75-inch net pots (Gro-Pro.net, Hawthorne Gardening Co. Vancouver, WA) filled with coconut coir (Green Grower Product of India, Tamil Nadu, India). Arugula was grown with either 0, 1, 2, or 3 oz/gallon of EM-1 (Tera Ganix, South Alto, TX) added to the recirculating nutrient solution. (0, 7, 14, or 21 total oz added in a 7-gallon bucket). This product was developed in Japan by Teuro Higa, with the main microorganism being *Lactobacillus casei*.

Six pots per treatment were set in a 1-inch thick styrofoam that had a reflective coating on top. This rested inside a round black 12.5-L container. A 4-inch weight was placed under the grow tray to increase the angle of the tray and help with drainage. Four containers were set on a greenhouse bench at the University of Florida, Fort Lauderdale Research and Education Center, Davie, FL. Plants were grown in an open-sided greenhouse exposed to ambient air temperatures, light levels, and relative humidity. We ran this experiment from Feb. 2020 to Mar. 2020. The average temperatures in the greenhouse were 26.1/17.2 °C (day/night). Light levels were approximately 184 W/m^2, and relative humid-

This work was completed as part of the senior author's MS degree at the University of Florida

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ity was 75.75%. Weather data were collected from the Florida Automated Weather Stations located less than 100 ft from the greenhouse <www.fawn.ifas.ufl.edu>.

Under the bench were four 7-gallon buckets. The lids had holes drilled in them to accommodate the tubes for the drainage, pump, and aerator. Inside each bucket was a standard fixed flow pump (ECO-185, Hawthorne Gardening Co., Vancouver, WA). The pump was attached to 2.96 ft of 3/8-inch diameter tubing to pump the nutrient solution into the round container. Each container drained by gravity through tubing connected at the opposite end of the in-flow tubing. The system was set on an automatic timer to deliver 1 inch of nutrient solution to the containers every 2 hours for 15 minutes. The solution then drains by gravity back into the buckets under the bench.

Each bucket contained 70 g of Maxigro 10N–5P_2O_5–14K_2O (General Hydroponics, Sebastopol, CA), which had 265 ppm N, 308 ppm K, 58 ppm P, 159 ppm Ca. Water was added to each bucket to bring it up to the 7-gallon volume. An additional 70 g of fertilizer was added at week 3 of the 6-week experiment. EM1 was added weekly to the nutrient solution at either 0, 1, 2 or 3 oz/gallon. Water was added to bring the buckets back to the original volume.

After 6 weeks, we harvested roots and shoots to measure dry weight. To determine the dry weight, shoots and roots were placed in a drying oven set at 145 °F for 7 days. We ground the dried shoot tissue using a Wiley mill (Thomas Scientific, Swedesboro, NJ). Tissue samples were dry ashed for analysis using an ICP-OES Avio 200 (Perkin Elmer, Waltham, MA). The ICP-OES measured P, K, Ca, Mg, Fe, Mn, Cu, Zn, and B in the shoot tissue.

We collected weekly nutrient solution samples to measure solution electrical conductivity (EC), nitrate (NO_3), potassium (K), and calcium (Ca). We used Horiba Scientific Laqua handheld meters (Horiba Instruments, Atlanta, GA) to measure NO_3, K and Ca and a HI 9813-6 portable pH/EC meter (Hanna Instruments, Smithfield, RI) to measure solution EC.

At week 6, we also counted lactobacillus colonies in the nutrient solution on plates that had no dilution, 10× dilution, or 100× dilution. We used a kang-fung-sol or KFS media created by a combination of MRS powder, with 1.75% phenyl ethanol and 1% lactic acid (Kim et al., 2007). The MRS media was added to 1-L milliQ water and autoclaved for 60 min. It was cooled in a water bath. The two other selective chemicals were then added. The pour plate method was utilized to have anaerobic and aerobic growth. The 200 uL sample was initially pipetted into the corresponding plate, and 10 mL of media was poured into the petri dish. The dish was gently swirled to mix the sample and the media. The plates were set aside for 15 minutes to harden and sealed with parafilm. They were flipped and put into anaerobic jars with an Anaero Pack sachet (Thermo Scientific R681001, Atlanta, GA). The jars were stored at 28°C in the dark, and after seven days, the plates are assessed to determine colony formation and make counts on the plates.

At week 6, we also collected 3 grams of root material to perform colony counts on roots. We used sterile petri dishes and sanitized scissors to cut roots from each replication. We weighed each dish on a tared scale to make sure we collected 3 g of roots. The roots were brought to the lab and suspended into 3 mL of milliQ water with 1 mL of KFS media. Once the roots were suspended, they were diluted from the original suspended solution to create 10x and 100× dilutions. Roots were collected before adding EM1 and 1 hour after adding EM1. Our culture medium and procedure were the same as used for solution analysis.

All data were analyzed by analysis of variance (ANOVA) using proc ANOVA in SAS. Differences between treatments were separated by Duncan's multiple range test.

Results and Discussion

The addition of EM1 did not increase shoot or root growth. There was no difference in shoot or root growth among the treatments (Fig. 1). Unfortunately, the benefit of adding microbes to the soil is often associated with improving growth by increasing the plant's tolerance to abiotic or biotic stress (Souza et al., 2015). When plants are grown under optimum conditions, the benefits of microbes are often masked or not observed.

Beneficial microorganisms often offer advantages such as improved nutrient uptake (Berg, 2009). We were curious to see if the addition of EM1 would improve nutrient uptake similar to the benefits seen by the addition of mycorrhizae in the soil (Bona et al., 2017). Microorganisms have the ability to make certain nutrients like N and P, more bioavailable for plants, whether applied as a mineral fertilizer or nutrients already in the soil (Bargaz et al., 2018). However, there was no difference among the four treatments for solution EC, NO_3, K, or Ca and we averaged these values to look at differences over time. Nutrient and EC levels were greater at week 6 than week 1 (Table 1). We did add more fertilizer at week 3 and observed that EC and nutrient levels were similar for weeks 4, 5, and 6.

There was no clear trend or evident benefit regarding nutrient uptake with increasing levels of EM1 in solution. For example, there was no difference in leaf tissue Mg, Zn, or B among the

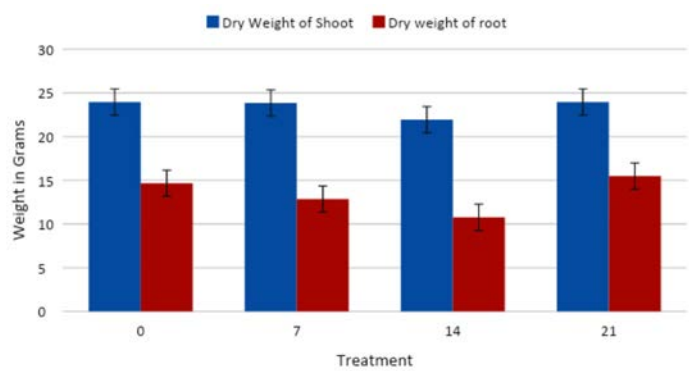

Fig. 1. Final arugula (*Eruca vesicaria* ssp. *sativa*) shoot and root dry weight of plants grown in ebb and flood system with 0, 7, 14, or 21 oz of EM1.

Table 1. Weekly solution values for electrical conductivity (EC), nitrate (NO_3), potassium (K), and calcium (Ca) levels in hydroponic solution. Arugula plants were grown in ebb and flood system treated with 0, 7, 14, or 21 oz of EM1. Values were averaged for the four treatments.

Week	EC (dS/m)	NO_3 (ppm)	K (ppm)	Ca (ppm)
1	3.9b	273b	470d	1140c
2	3.9b	258b	698c	1200c
3	5.4a	343ab	1525b	1825a
4	5.3a	498a	1825a	1800a
5	5.0a	450a	1875a	1863a
6	5.1a	555a	1850a	1825a

Means followed by a different letter are not significantly different at the 5% level as measured by Duncan's multiple range test.

treatments (Tables 2 and 3). Tissue K was greatest in plants treated with 7 oz, while the greatest tissue Ca was in plants treated with 0 oz (Table 2). Tissue P and Fe were greater with 0, 7, or 14 oz than with 21 oz (Table 2 and 3). This is unlike the study conducted with cucumbers (*Cucumis sativus*) treated with the *R. sphaeroides* that had a higher P, Ca, Mg, and K content in treated plants than control plants (Kang et al., 2015).

It is a known phenomenon for microbial inoculants to decrease after being added to the substrate because of poor production of bacterial biomass, difficulty sustaining in the rhizosphere, and possible competition between microorganisms (Bashan et al., 2014). Most rhizospheres can contain up to 100 million to 1 trillion microorganisms in a single gram (Kennedy and de Luna, 2005). However, the hydroponic rhizosphere is suspected to be less diverse than soil, so the effects of adding microorganisms into the nutrient solution might be limited.

When the EM-1 was plated on the KFS media, it corresponded with the printed label of 1 million CFU/oz. However, at week 6 there was no difference in CFU among the 7-, 14-, or 21-oz treatments (Fig. 2). At the end of week 6, we had roughly only over a few million CFUs compared to the hundreds of millions of colonies we started with at the beginning of week 6. Observations of the roots led us to suspect that lactic acid bacteria (LAB) were staying on the roots. We observed an increased number of CFU on the roots. After 6 weeks, the greatest number of CFU on the roots was for the plants grown with 21 oz. of EM1 (Fig. 3). We suspect the arugula roots might have been releasing a photosynthetically derived carbon to have such high colony counts on the roots (Mendes et al., 2013).

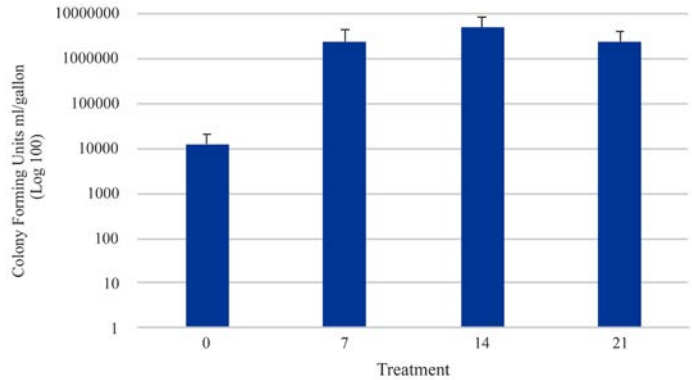

Fig. 2. Colony-forming units per gallon of solution at week 6. Arugula (*Eruca vesicaria* ssp. *sativa*) plants were grown in an ebb and flood systems with 0, 7, 14, or 21 oz of EM1 added to the nutrient solution.

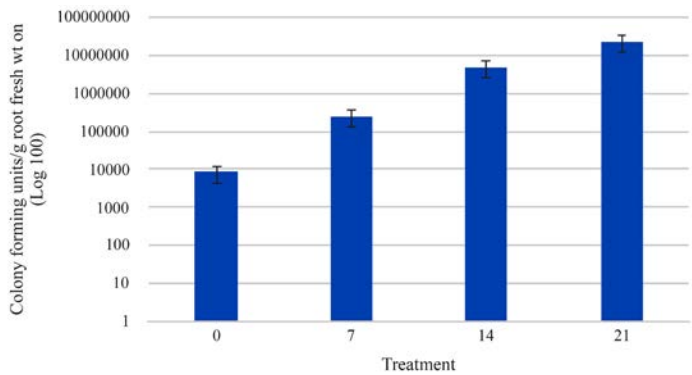

Fig. 3. Final colony forming units per gram of arugula (*Eruca vesicaria* ssp. *sativa*) total root fresh weight at week 6. Arugula plants were grown in an ebb and flood system with 0, 7, 14, or 21 oz of EM1 added to the nutrient solution.

Table 2. Final leaf tissue phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) concentrations for arugula (*Eruca vesicaria* ssp. *sativa*) plants grown in ebb and flood system treated with 0, 7, 14, or 21 oz of EM1.

EM1	Arugula (*Eruca vesicaria* ssp. *sativa*) leaf tissue concentration (ppm)			
treatment (oz)	P	K	Ca	Mg
0	54.55a	6621.b	210.7a	69.26a
7	54.17a	873.5a	185.2b	64.27a
14	51.66a	770.6ab	178.3b	72.02a
21	41.28b	746.8ab	159.3b	69.53a

Means followed by a different letter are not significantly different at the 5% level as measured by Duncan's multiple range test.

Table 3. Final leaf tissue iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), and boron (B) for arugula (*Eruca vesicaria* ssp. *sativa*) plants grown in ebb and flood system treated with 0, 7, 14, or 21 oz of EM1.

EM1	Arugula (*Eruca vesicaria* ssp. *sativa*) leaf tissue concentration (ppm)				
treatment (oz)	Fe	Mn	Cu	Zn	B
0	0.51a	0.69b	0.04ab	0.28a	0.86a
7	0.44ab	0.55b	0.02c	0.29a	0.71a
14	0.51a	1.05a	0.03bc	0.31a	0.69a
21	0.35b	1.05a	0.05a	0.35a	0.68a

Means followed by a different letter are not significantly different at the 5% level as measured by Duncan's multiple range test.

Conclusions

It was clear that EM-1, at the application rates used, did not improve arugula yield. There was no difference in nutrient levels in the hydroponic solution between the treatments suggesting that the addition of LABs did not improve nutrient uptake. Minimal differences in tissue nutrients levels supported this. Improved growth with the addition of microbes is often observed when plants are exposed to abiotic and/or biotic stress. However, when plants are grown under optimum conditions, the benefits of adding microbes may be often undetectable.

As we began plating the nutrient solution, we expected to see high amounts of CFU's in the solution. However, we observed no difference in CFU's in the solution among the treatments. When we looked at the roots, we noticed an accumulation of a brown biomass on the roots and differences in root appearance among the treatments, which was supported by greater CFU found on the roots. It is unclear why the colonization of the roots with LAB did not improve growth compared to the control.

LAB's have the ability to form biofilms and produce a variety of BCA's like antifungal diketopiperazines, hydroxy derivatives of fatty acids, 3- phenylacetate; antibacterial bacteriocins and bacteriocin-like compounds, and general antimicrobials such as organic acids, hydrogen peroxide, pyrrolidone-5-carboxylic acid, diacetyl and reuterin (Lamont et al., 2017). LAB's might improve

plant growth under high levels of salinity as well as improve plant response to diseases like *Pythium*.

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Restoring a 1920s Golf Course Into an Ecological Park: A Case Study of the Rolling Hills

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The Rolling Hills Park project is a Florida Communities Trust (FCT) grant to convert a non-operational golf course to a passive park. Seminole County purchased the 98-acre golf course in August 2018 and maintains the property. The goal of this repurpose project is to apply sound urban ecology design principles to the development of a nature-based recreation park and urban conservation habitat. Through partnership funding from the University of Florida facilitated by the Seminole County extension agent, three students (one undergraduate and two graduate) developed a plan for the park in Summer 2019. Project aspects included: 1) selection of commercially available plant species; 2) total site soil testing; 3)principles for creating habitat; 4) coexisting with wildlife; and 5) a master plan of plant communities and recreation amenities to foster a visually pleasing, urban conservation space. Under the direction of the extension agent and other advisors the students created a Rolling Hills handbook, which outlined the details of their recommendations. At the end of the semester the results were presented to Seminole County staff, decision makers and University of Florida advisors. The county staff was very excited about this work and has since integrated the plan into their master-site planning and implementation. Later in 2019 it was provided to county consultants and other key players in the project. The students used the urban ecological model to address both human activities and conservation needs. They did it in a way that was practical and helpful to the county staff, who were grateful and recognized the value of their work at over $30,000. Additionally, their diligent work will be incorporated into the implementation of the park as the plans are funded annually through the county's capital improvements budget. The Seminole County extension agent will continue to work and collaborate on this project.

Florida-Friendly Landscaping™ (FFL) means using low maintenance plants and environmentally sustainable practices. To achieve a Florida-Friendly Landscape™ , the 9 FFL principles should be followed during landscape design and management. FFL landscapes create habitat for wildlife while also lessening a sites footprint on the surrounding ecological community. For more information on FFL principles visit <https://ffl.ifas.ufl.edu/>.

Seminole County obtained and desired to restore a publicly accessible golf course, and this presented an opportunity to incorporate FFL. Improving urban and degraded areas while embracing smart choices in design will benefit the site and community for years to come. Increasing green space and utilizing green infrastructure has immense benefits that improve well-being, society, and global biodiversity. Placing a monetary value on ecosystem services has been a method adopted by many organizations to display the worth that comes from their landscape. Our goal in this project was to marry visitor usability with real restoration that would foster habitat for many species. We began with soil samples for the entire site and analysis.

Collection methods

Composite samples were composed of 10 different samples selected in a stratified random fashion. Locations were chosen based off their potential to create a representative profile of the specific section. To accomplish this, three composite samples were done per parcel; two composite samples per fairway and one composite sample per putting green. Soil probes were used to collect the soil from the upper six inches. The ten representative samples were placed into a bucket, mixed and transferred into the soil test package. All 50 samples were mailed to the University of Florida IFAS Analytical Services Laboratory to test for soil pH, lime requirement, macronutrients and micronutrients

Results

Soil type—Myyaka. Myyaka soil is Florida's State soil. It covers a majority of the state, about 1.5 million acres.

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Soil texture—sandy. Sand can be characterized as loose, single-grained, gritty to the touch, and easily seen and felt. When sand is moistened, it will perform a weak cast and crumble to touch.

General considerations of sandy soils—due to sandy soil's decreased surface area and increase pore space, it presents several unique characteristics that should be considered in landscape management. Sandy soils tend to be low in organic matter and native fertility, low in capability to retain moisture and nutrients, low in cation exchange and buffer capacities, and permit rapid movement of water and air (Pearson, 2013).

Soil pH for the entire site is not one for concern. The median soil pH is 6.1 in Florida, characterized as slightly acidic. According to University of Florida IFAS recommendations, if the soil pH is between 5.5 and 7.0 there isn't a need to adjust pH for landscape plants (Shober et al.2008).

The average pH for the fairways was 5.96. Putting greens on average were much more acidic, the median 4.98. These soils can be amended if time and money permits, but there are acid-loving plants that do well in pH values less than 5.5 (Anon., 2015).

Soil macronutrients

Results received from the soil laboratory indicate two irregularities amongst phosphorous and magnesium levels. This was due to differences in soil texture and past maintenance. Soil on greens was relatively more sandy and varied in macronutrient levels. This is consistent with the golf industries methods for turfgrass management. Soil levels are compared against Mehlich-3 interpretation levels. The Mehlich-3 interpretations provide a baseline to delineate between macronutrient levels in acidic soils (Mylavarapu et al. 2014).

The sand-based putting greens have lower levels of nutrient-holding and buffering capabilities. Reduction in buffering capabilities decreases the resistance the soil has to maintain a constant pH. With the intensity at which the putting greens were managed, the pH of the soil was highly influenced relative to fairways/historical soil. Putting greens are less than 2% of the square footage of golf courses; yet typically other turfgrass on golf courses are not as intensively maintained (Schlossberg, 2006). Sandy soils combined with prior management resulted in the increased acidity of the putting greens throughout the course.

Calcium (Ca) levels were adequate throughout Rolling Hills. Florida soils are naturally high in Ca because much of the parent material of Florida soils is composed of limestone (calcium carbonate, $CaCO_3$) (Shaddox, 2018).

Fertilizer additions will need to be done to supplement low potassium (K) levels. Sandy soils in Florida have low K retention. The results indicated here are not abnormal. Amendments simply require fertilizer application. Smaller and frequent doses should be done during application.

It is typical in Florida soils for there to be sufficient levels of phosphorous (P). Levels at Rolling hills were higher because of historical fertilizer additions containing P. Phosphorous does not leach out of soil easily relative to other macro-nutrients. There will not be a need to apply P in the near future until levels decline.

Magnesium (Mg) levels were adequate in all of the soils, though the putting greens barely fell within base level recommendation because of prior intense management practices.

Soil micronutrients tested included copper (Cu), manganese (Mn), and zinc (Zn). The results were adequate and did not indicate additional fertilization was necessary. These results are preliminary indication and observation should be given to individual plants and plantings to look for sign of deficiency.

Discussion

Fertilizer should be applied where needed. It is suggested that a high percentage of fertilizers used should be slow or controlled release rather than water-soluble quick release fertilizers (Q.R.F.). The latter are not suitable for a site like Rolling Hills. Sandy soils will cause the Q.R.F. to leach before the nutrients can be absorbed by plants.

Slow and controlled release fertilizers are the better alternatives because they decrease nutrient losses, enhance nutrient-use efficiency, and require less frequent application. Selecting slow and controlled releases fertilizers can decrease fertilizer use by 20 to 30% of the recommended rate of a conventional fertilizer while obtaining the same yield (Zotarelli et al. 2017). Fertilizers should only contain N and K, the level of P currently in the soil is sufficient. Additional applications of P can cause leaching from the soil into ground water.

To increase Mg levels on putting greens dolomitic lime can be used. Dolomitic lime contains Mg and will also increase the pH. If soil amendments are desired, then Mg will be needed to be added to fertilizer. Long term, the best solution would be amending putting greens with dolomitic lime prior to planting; again, this will increase the pH in acidic areas and raise needed Mg levels.

Rolling Hills master plan

Subsequent pages of the report go through each of the areas laid out on the master plan. Each section highlights a proposed landscape design with explanations on elements to consider including viewpoints, topography, pedestrian access, soil profiles, recommended habitat features, anticipated wildlife and plant selection tips.

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—Scientific Note—

Homeowner Perceptions of Florida Wildflowers for Ecological Landscaping

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Additional index words. biodiversity, ecological landscapes, Florida-Friendly, residential landscapes, wildflowers

The biodiversity of flowering plants that once existed in Florida's natural areas has been reduced as urbanization has converted these areas into housing and retail developments. The diversity of wildflowers and native plants is critical to supporting other native species but they are often lost and rarely replanted in residential landscapes. These native plants have co-evolved with native insects and aid in the support of unique pollinators that ornamental landscapes do not support. These flowers can also help with pest control in the home landscape by attracting beneficial predatory insects that prey on landscape pests (Dale et al., 2020). Residents face barriers to adding native diversity to their landscapes because native plants and wildflowers are hard to find at local retail outlets in central Florida. One way to overcome this is by using wildflower seeds, since they are cheap and can be ordered online and delivered to your house. The objectives of this study were 1) to determine consumer preferences of five wildflower species for cultivation in home landscapes in central Florida and 2) to identify educational needs of residents that can be provided by extension agents to help them establish wildflower plantings in their yards.

Five wildflower species were direct seeded at the Orange County Extension Center in Orlando, FL. Species included two species of tickseed (*Coreopsis lanceolata* and *C. leavenworthii*), blanketflower (*Gaillardia pulchella*), phlox (*Phlox drummondii*), and milkweed (*Asclepias tuberosa*). Landscape plots 1.2 × 1.2 meters in area were prepared by loosening the soil with a hoe, leveling it out with a rake, and sprinkling the seeds on the ground and patting them in on 1 Oct. 2019. The experimental design was set up as a randomized complete block design with 4 replications. Plots were maintained with hand weeding and watered twice a week for establishment during the first month and once a per week after establishment. No fertilizer or pesticides were used although yellow aphids were found on the milkweed. A field day was conducted on 20 Feb. 2020 that included an educational PowerPoint presentation, followed by a tour of the wildflower trials and planting demonstration. A consumer preferences survey was administered to the people who attended the field day.

Questions on the likelihood to grow wildflowers at home, wildflower species preferred, and where to buy plants and seeds were asked. Additional outreach events were conducted by giving a virtual tour of the wildflower trial on Facebook and walk in visitors were also surveyed.

Results showed 80% of respondents (n = 32) indicated they would be extremely likely to grow wildflowers at home. Blanketflower was ranked by 46% of respondents as the number one choice for most likely to grow. Phlox and one of the tickseed species (*C. lanceolata*) were also in the top three most likely to grow choices. Mixed responses were reported for milkweed, which was ranked as both desirable and undesirable. Milkweed is well known as a host for monarch butterflies however it did not score highly in this study. When asked "where would you be willing to go to buy seeds/plants?", 72% of respondents indicated they would order seeds online from the Florida Wildflower Seed Cooperative, which had been the seed source for this study. Buying plants from a local native nursery and buying seeds/plants at garden festivals, were ranked second and third, respectively. Saving seed from their wildflowers in their yard was well received. The least popular method to obtain plants and seeds was order from other online catalogs, most likely because commercial catalogs do not commonly carry Florida native wildflowers.

Native plants and wildflowers need to be part of a Florida-Friendly Landscape as they are adapted to low inputs of fertilizer and can withstand rainfall and drought. Overall, residents indicated interest in growing many species of wildflowers. More extension resources are needed to help residents cultivate wildflowers in their yards on the topics of seed planting, seedling versus weed identification, maintenance, pest control, seed saving and replanting. Educating people to understand that increasing our native plant diversity would also cause an increase in the diversity of native birds and pollinators and also provide food and shelter for wildlife will also be key to creating more eco-friendly home landscapes.

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—Scientific Note—

Plant Diversity Across Agroecosystems Representing Different Disturbance Regimes In South Florida

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Additional index words. agroecology, botany, biodiversity, flora, NEON

South Florida has diverse plant species and land uses. Both the environment and management by humans drive the existence and assemblage of plant species. Adjacent lands frequently differ in their use and management throughout south Florida. Different habitats emerge from the dispersal and survival of diverse plant species. We examined how habitat and tillage history affect plant species richness and diversity at an agricultural research center in southern Florida. The objective was describing how plant species assemble into closely arranged yet distinct habitats under different cultivation and management regimes. Plant species in cultivated farming systems differ according to whether the land was untilled lawns or tilled fields and for how long the tilled fields had been cultivated (3 years or > 20 years).

The plant survey was conducted at the University of Florida Tropical Research and Education Center (TREC) near Homestead, FL. The site experiences a wet season from May to October, mean annual temperatures of 23.3 to 24.0 °C, and an annual average 56 inches precipitation. TREC soil is high pH, well-drained, shallow, rocky, and uniformly non-flooded. The plant survey used sampling protocol from the National Ecological Observatory Network (NEON, Barnett 2017). We identified plant species in four pairs of 20- × -20 m sampling plots. Four plots were located in fallow agricultural blocks with two per tillage history. The old agricultural plots had been tilled for at least 20 years, while the new agricultural plots were tilled for the first time in 2018. Soil tests indicated that the old plots had significantly less organic matter than the new plots. Each agricultural plot was paired with a nearby (< 150 m) lawn plot.

Plots were set up and surveyed in Mar. 2020. Within each plot, nested subplots were arranged according to NEON protocol and included eight 1 m^2, eight 10 m^2, and four 100 m^2 subplots. All plant species located within each subplot were identified. Subplots were monitored through 30 June to identify any plant species that were ambiguous during the survey period. Species richness and assemblage diversity were compared across subplots, habitat and tillage history.

The plant survey identified 126 unique plant species (36 families). Species richness increased across subplot size for all plots but did not appear to be different across tillage history or habitat. Richness in the new agricultural plots tended to be higher than in old agricultural plots. Assemblage diversity clustered distinctly for 10 m^2 subplots grouped by old and new agricultural plots and by agricultural and the lawn plots as determined by a non-metric multidimensional scaling analysis. Assemblage diversity for the 10 m^2 subplots was more similar among plots, tillage history driven by the agricultural sites, and habitat, respectively, as determined by permutational multivariate analysis of variance [(PERMANOVA), Anderson, 2001]. The results suggest that tillage history, and perhaps soil organic matter, led to different, but not more species-rich, plant assemblages. These agricultural plant assemblages were also distinct from untilled lawn plots nearby.

Studies of plant diversity across habitats will continue to examine the effects of land use, land management, and landscape species richness and diversity within agricultural and natural areas.

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—Scientific Note—

Experiential Urban Forestry Extension: Treejuvenation Florida

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A Sarasota County canopy study in 2013 indicated 35% vegetation cover in the Urban Service Area. With increasing land development, this canopy has steadily decreased. Urban trees provide significant community benefits, including reduced heat-island effects and improved air quality. To increase urban forestry community engagement and support canopy conservation efforts, an urban forestry extension program—Treejuvenation© Florida—was developed in Sarasota County.

A series of quarterly urban forestry community engagement projects were conducted. These projects were designed to increase community awareness of tree assets, increase community engagement in urban forestry activities, increase tree plantings, and identify urban forestry partnership resources. The projects included: *TreeQuest*—scavenger hunts for native trees; *Talking Trees*—urban forestry youth workshops; *Tree Trail Tour*—tree-centric nature walks; and *Adopt-a-Tree*—tree planting demonstrations and seminars. As part of the urban forestry extension program, a partnership with New College, a local college was also launched, providing assistance with completing the certification process for the college to be designated as a Tree Campus USA© site.

TreeQuest is an outdoor experiential learning activity designed to promote community awareness of urban tree benefits and hosted on Florida Arbor Day. For the event, native trees in local parks and preserves are marked with "clues" on an 11 × 17 inch vinyl tag, which includes information highlighting benefits of urban trees such as the estimated amount of storm water filtered annually, and lifetime of carbon dioxide sequestered. This information is derived from the iTree©/MyTree© Benefits database. Scavenger hunt participants submit clues online for a prize.

Arbor Day Adopt-a-Tree is a community engagement activity designed to increase the number of tree plantings in Sarasota County. Tree-planting demonstrations and tree adoption events are hosted on National Arbor Day. After attending an urban forestry seminar on tree care, participants pledge to plant a tree on their property and receive up to 3 native trees per local address. To assess new tree survival rates, a follow-up survey is done with participants 1 year after the tree-planting event, with participants having the option of submitting pictures of their newly planted tree(s) in the survey response.

Talking Trees is a series of summer workshops designed to educate youth about the ecosystem services of urban forests. Using experiential learning activities from the Project Learning Tree Curriculum, youth learn about the roles of trees in the carbon cycle and supporting wildlife habitat.

Tree Trail Tour is a series of tree-centric nature walks in local gardens and arboreta, designed to increase urban forestry community engagement, and is hosted in recognition of National NeighborWoods month. Copies of plant maps are distributed, and participants embark on interpretive tours highlighting the urban forestry benefits of specific trees.

Tree Campus USA©: To increase college student engagement in urban forestry activities, the *Treejuvenation© Florida* Extension program initiated a partnership with New College, one of Sarasota's colleges, assisting the college to complete the Tree Campus USA© certification eligibility process. Colleges with a Tree Campus USA© designation are annually required to commit staff, student and financial resources to promoting urban forestry awareness on campus, and engaging students in urban forestry activities.

TreeQuest: Feedback from participants over a 3-year period (n = 151) showed that 94% of respondents increased their knowledge about the benefits of urban trees; 83% indicated that they would share the information they learned with others; 61% indicated that the learning activity motivated them to plant a tree.

Adopt-a-Tree: Treejuvenation Florida hosted 11 tree adoption stations over a 3 year period with 355 trees adopted. A one-year post-planting follow-up survey to measure the survival rate of the trees, showed an 84% survival rate (300 trees).

Talking Trees: Ninety percent of the urban forestry youth summer workshop participants indicated that they would share what they learned about trees with others.

Tree Trail Tour: Ninety-eight percent of participants indicated that they learned a lot about urban tree benefits, and they would share what they learnt with others.

Tree Campus USA©: In collaboration with the *Treejuvenation© Florida* Extension program, New College of Florida successfully completed certification requirements for designation as Florida's 21st Tree Campus USA© location.

Since its launch in 2017, *Treejuvenation© Florida* has promoted awareness about the benefits of urban trees, conducted 31 urban forestry extension learning activities, with 421 participants planting 300 native tree trees. With a mature native tree having a lifetime capacity to sequester more than 3000 lbs. of carbon-dioxide-equivalent of carbon, these additional tree plantings represent a carbon sequestration potential of over 900,000 lbs. of carbon.

Treejuvenation© Florida's project and resource partners include: Andrew Koeser, Environmental Horticulture Department, University of Florida; Brad Oberle, New College of Florida; Sarasota County Extension Master Gardener Volunteers; Sarasota County Parks and Natural Resources Department; Sarasota County Library Department; Project Learning Tree; and iTree© My Tree Benefits database.

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Calcium management and the usage of organic acids: Impacts on marketable fruit yield in strawberries

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Introduction

The Florida strawberry (*Fragaria x ananassa*) market is estimated to have a production value around $337 million; comprising roughly 10% of total US production (FDACS, 2017). According to the Agricultural Marketing Resource Center, 82% of production from of this nearly $350-billion US-industry is in fresh market sales (AgMRC, 2019). Fresh market strawberries are particularly susceptible to metrics that negatively affect fruit ship ability (i.e fruit firmness and shelf-life). Influence of disease and certain environmental pressures can thus have significant economic impact.

In the past decade, the primary strawberry production area of Central Florida has seen increases in both temperature and solar intensity. The winter months of December, January, and February are peak months for strawberry market and harvest in Florida. The 2019-2020 season marks the ninth consecutive winter with mean temperatures above normal (NOAA, 2019). According to the National Weather Service (NWS), 2018-2019 season in Plant City, FL averaged +3.9°F above the historic mean, making it the sixth warmest winter on record (NOAA, 2019). February of this same season averaged +8.7°F above the historical mean, making it the second warmest February on record for the area (NOAA, 2019). Data from the NWS shows that for this geography, the number of days per year that the Ultra-Violet (UV) Index was recorded as *very high* or *extreme* has increased from 143.8 days (5-year avg. 2003-2007) to 189.4 days (5-year avg. 2013-2017) (NOAA, 2017).

Increased exposure to high temperature and intensive solar radiation are known to negatively impact the commercial quality of fruit (Rosales et al, 2009). In the past several years as environmental influences continue to trend in this direction, reports from growers and brokers on issues of ship ability have become more predominant. Because of the large potential for economic loss in fresh market strawberries, it is suggested that adjustments to production methods should be considered. Placing an increased importance on sustainable management

for calcium (Ca) as a primary nutrient related to cell wall structure may provide a strategy to improve the percentage of marketable fruit with adequate shelf-life. This publication provides an overview of science-based information on the role of calcium in fruit storage and the rhizosphere interactions that can limit Ca-uptake. A field-study is used to investigate how the presence of organic acids as a soil amendment could be a potential option for improving the concentration and mobility of this target nutrient in developing fruit.

Calcium Role in Fruit Storage

Fruit firmness is a quality component directly associated with cell wall structure and is economically important to shelf-life of fresh market produce. The primary structural strength of the cell wall comes from cellulose fibers bound together by pectic strands in the middle lamella. A majority of the pectic material found in plants is of the form calcium-pectate (Maschner, 1995). Natural processes for ripening involve ethylene-signaled pectinase enzymes (i.e. polygalacturonases) solubilizing pectin in the cell wall of the fruit to create a softer, more edible berry (Pombo et al, 2008). High Ca-concentration in plant tissues has been directly linked to the inhibition of polygalaturonase activity and thus a reduction in the rate of cell wall disassembly (Corden, 1965). Although the biochemical pathways involved with cell wall modification and fruit ripening are complex, they exemplify the significant role calcium plays in maintaining cell wall integrity.

The degradation of structural pectins, however, is not solely a plant-regulated process. Dr. Rosales and her colleagues (2009) associated an increase in the solubilization of pectins and the loss of commercial quality in cherry tomatoes with a reduction in Ca content and an increase in oxidative stress caused by temperature, solar radiation, and vapor-pressure deficit. Morales-Quintana et al (2019) elaborated by stating that the disassembly of cell wall components due to high temperatures and certain wavelengths of solar radiation is primarily explained by the biochemical increase of photo-oxidative species within the plant during such climatic conditions. Cellular oxidation is a term used to describe the process by which cell membranes lose their structural integrity. The consequential leakage of low-molecular weight substances (i.e. sugars and amino acids) from the cell via lost cytoplasm can quickly become substrates for secondary pathogenic infection; further inhibiting plant health and agronomic production (Dordas, 2008). A common example of this is the contraction of the fungal species *Botrytis cinerea*, often exhibited in late season strawberries and evident by a fruit-rotting gray mold on ripening berries. Naradisorn et al (2006) observed a reduction of *Botrytis cinerea* incidence and an increase in storage life of strawberries when pre-harvest calcium was applied. In summary, inadequate calcium concentration in the plant, and the consequential weaknesses to cell wall structure, can negatively affect both plant health and volume of marketable fruit. In adverse conditions of high temperature, intense solar radiation, or additional nutrient deficiencies, consequences are magnified.

Limitations to Calcium Mobility

Managing a crop for adequate calcium has challenges, primarily related to the limitations of plant acquisition and nutrient mobility. Being a divalent cation (Ca^{+2}), calcium forms a variety of strong bonds with other soil components, often rendering it inaccessible for plant uptake. Below a pH of roughly 5.5, Al^{+3} and Fe^{+2} become more available in soil solution, thus inhibiting Ca^{+2} uptake (Taylor et al, 2004). Above a pH of roughly 7.5 and calcium begins to form insoluble precipitates with phosphorus (Alvarez et al, 2004). Even within the optimal pH range of 6.0-6.5, plant uptake processes for other essential nutrients (i.e. K^{+}) can compete with Ca^{+2} acquisition (Taylor et al, 2004). Although Ca^{+2} uptake is regarded as a passive diffusion process, it is still subject to transpiration forces and the pull of water through the plant (Taylor et al, 2004). Low water availability, high relative humidity, restricted root growth, and low temperature are all factors that inhibit transpiration and thus calcium movement (Taylor et al, 2004).

Once in the plant, calcium is heavily restricted to uni-directional movement in the xylem (Taylor et al, 2004). Depending on bond strength of the calcium carrier and number of active exchange sites within the cell walls of the xylem, calcium distribution becomes quite complex (Marschner, 1995). Once established in a specific tissue, calcium translocation from one plant organ to another through the phloem is limited (Marschner, 1995). In addition, plant tissues with higher transpiration rates (i.e. leaf surfaces) become calcium sinks (Marschner 1995). In agricultural systems, this has the potential to disproportionally draw calcium away from high growth rate, low-transpiring fruit organs (Marschner, 1995). This aspect of calcium mobility greatly increases the risk that calcium concentration in developing berries falls below the critical level required for adequate cell wall stabilization (Marschner, 1995).

Calcium as Natural Resource in Florida Soils

Much of the parent material that geologically formed Florida soils is limestone, solidifying calcium carbonate as a feature in the majority of Central Florida's cultivated ground (Bostick et al, 2018). Additionally, Central Florida soils are characteristic of having primeval deposits of phosphate rock (FIPR, 2020). An early geological survey of these deposits by the Department of the Interior indicate that the predominant composition of these phosphate rock sources is in the form tricalcium-phosphate ($Ca_3P_2O_8$)(Matson, 1915). As a result of these geological attributes, calcium is found in relatively high quantities in Central Florida soils. Recent soil samples taken on cultivated ground across the Plant City, FL strawberry production area indicated an average soil concentration of extractable calcium at 1766 lbs Ca/ac (Mehlich I). Despite these relatively high levels, limitations to calcium uptake and plant integration could still inhibit certain crop species from producing fruit with calcium concentrations adequate to counteract adverse environmental conditions.

Calcareous soils are generally characterized by low bioavailability of plant nutrients (Strom et al, 2005). As a result, calcicole plants, or plant species that thrive on calcareous soils, have been documented to exhibit increased organic acid root exudation, particularly of oxalic and citric acids (Strom et al, 2005). Based on the soil samples taken from the Plant City, FL strawberry production area, low clay content (mean: 95.3% sand), low organic matter (mean: 1.0%) and low cation exchange capacity (mean: 7.3 meq/100g) suggest a strong likelihood that a large portion of this extractable calcium source is 'tied-up' in low-availability forms (i.e. calcium carbonate, calcium phosphate, etc.) as opposed to being bound at the soil exchange complex. Management practices aimed at increasing solubility of unavailable nutrients for agronomic use are needed.

Role of Organic Acids

Soil organic matter (SOM) plays a centric role in soil health, fertility, and nutrient cycling. Humic acids are a form of high-molecular weight (HMW) organic acids often applied as an amendment to increase agricultural productivity on soils with weak structure or low CEC (Delgado et al, 2002). Commercial production of humic acid is typically derived from naturally occurring carbon deposits of lignite and leonardite. The addition of humic acid has chemical, physical, and biological impacts on the soil (Pettit, 2007). Chemically, the addition of humic acid increases the number of nutrient exchange sites, particularly for cations such as calcium (Pettit, 2007). The negatively charged hydroxyl and carboxyl active groups on the humic colloid are involved with binding and holding cationic nutrients in plant exchangeable forms (Pettit, 2007). In addition, the presence of humic acid has been noted to increase the movement of nutrients into the root system by upregulating active transport proteins (i.e. H^+-ATPase) embedded in the root plasma membranes (*Table 1*)(Pinton et al, 2009).

Root exudation is a remarkable process by which plants alter their below-ground environment for more suitable growth and homeostatic balance (Badri et al, 2009). Plant to soil, plant to microbiota, and plant to plant signaling are symbiotic relationships regulated by root-secreted compounds (Haichar et al, 2014). Evidenced by the high cost of photosynthate, with 5-21% of photosynthetically fixed carbon secreted into the rhizosphere, root exudation is a physiological process of great importance for plant success (Haichar et al, 2014, Strom et al 2005).

Low-molecular weight (LMW) organic acids are a classification of root exudate with many roles but are commonly associated with nutrient exchange (*Figure 1*). In the case of organic acids, a proton (H^+) dissociates and replaces the target cation on the colloidal exchange site. The anion portion of the organic acid then complexes the target cation to keep it from taking a new, potentially insoluble form (*Figure 2*). For the case of calcium phosphate, it has been suggested that organic acids produced in the rhizosphere drive the dissolution of $CaPO_4$ by supplying adequate protons (H^+) to displace and bind Ca^{+2} ions (Bolan et al, 1994). Strom et al (2005) documented that at a pH of 7.5 in a calcareous soil solution, citrate and oxalate were the

organic acids most effective at solubilizing phosphorous while citrate and malate were the most capable extractors of calcium. Citric acids are also associated with the dissolution of certain carbonate minerals (i.e. $CaCO_3$) resulting in the net release of cations into solution (Jones et al, 1994). When studying the behavior of organic acids in calcareous soil, Strom et al (2005) also noted that the dissolution of $CaCO_3$ by organic acids resulted in the formation of organically-complexed calcium (i.e. calcium-citrate, calcium-oxalate, etc.) and in increase in soil pH explained by the release of carbonate ions (CO_3^{-2}). Importantly, the newly formed organo-calcium complex has also been observed to have improved mobility once in the plant. Plants with higher concentrations of organic acids in the vascular tissues are reported to have wider distribution of calcium throughout the plant (Marschner, 1995).

Investigative Field Study

A non-replicated field study to explore the application of humic and organic acids (HA+OA) as effective soil amendments was performed at three farm locations in Plant City, FL during the 2019-2020 season. At one of these locations, a replicated harvest was performed once a month during peak season (December, January, and February) on strawberries (cv. *Brilliance*) to establish fruit quality metrics.

The data retrieved shows an early and mid-season trend towards increased concentration of calcium in soil solution (*Figure 3*). While the combined response of all three farm locations to the HA+OA treatment was non-significant ($p=0.181$), a single-site location considered as "low-input" did have a significant response ($p=0.041$). Despite these minor increases to concentration of calcium in soil solution, there were not significant changes to calcium concentration in the leaf-tissue. Furthermore, the replicated berry harvest did not exhibit significant changes to calcium concentration within the fruit-tissue nor in fruit firmness. Increases in the number of marketable berries and the weight per marketable berry were, however, observed in the HA+OA treated plots (*Table 2*). It should be noted that no treatment included additional units of calcium so any observed differences are recognized as the solubilization of calcium-containing compounds in the soil by applied organic acids.

The nutritional analysis of soil solution also presented an unexpected but significant ($p \leq 0.05$) difference in soluble phosphorous (P) between treatments (*Figure 4*). This further suggests the dissolution of phosphate rock deposits by organic acids as a primary mode of action for the release of P and Ca into solution. The addition of HMW and LMW organic compounds to the soil may increase the efficacy of applied P fertilizer in calcareous soils by reducing P adsorption and ultimately serving as an effective crop production tool (Delgado et al, 2002). Despite the observed difference in P-solubilization due to organic acid amendment, there were no significant differences in leaf- or fruit-test P between treatments.

Conclusion

With a landscape of changing climatic influences and a market dominated by fresh sales, production strategies that mitigate environmental variability and positively impact fruit quality in strawberries should be considered. This publication outlines the importance of calcium to cell wall integrity, strawberry shelf-life and how organic acids interact with soil-bound plant nutrition. The literature consistently supports that even relatively small increases to Ca concentration in fruit can be an effective method for improving marketable yield, limiting fungal infection, and avoiding storage disorders that result in economic losses (Singh et al, 2007). Data provides evidence that the addition of organic acids could be promoting the dissolution of calcium and phosphorous from the phosphate rock and lime rich soils of Central Florida, particularly effective in low-input agricultural systems. While the HA+OA amendment did not significantly change fruit firmness, there was a numerical increase of marketable fruit yield. It should be noted that the growing conditions of the 2019-2020 season in Plant City, FL exhibited ideal temperature and precipitation, and a scenario of more extreme circumstances may impact those findings. Further investigation of these concepts is needed to establish a stronger trend.

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***Table 1**: "Effect of Different Humic Substances Fractions (HS) on Active Proton Extrusion from Intact Roots and on H^+-ATPase Activity of Plasma Membrane (PM) Vesicles Isolated from Different Plant Roots" (Pinton et al, 2009).*

	Control	+HS
H^+ release from intact roots[a]	14 ± 1	35 ± 3
(nmol H^+ g^{-1} FW)	(100)	(250)
PM H^+-ATPase activity		
ATP hydrolysis[b]	95 ± 4	122 ± 4
(μmol Pi mg^{-1} prot h^{-1})	(100)	(128)
ATP hydrolysis[c]	74 ± 5	152 ± 8
(μmol Pi mg^{-1} prot h^{-1})	(100)	(205)
ATP-dependent H^+ transport[c]	17.4 ± 1.1	27.0 ± 2.1
(ΔA_{492} mg^{-1} prot h^{-1})	(100)	(155)

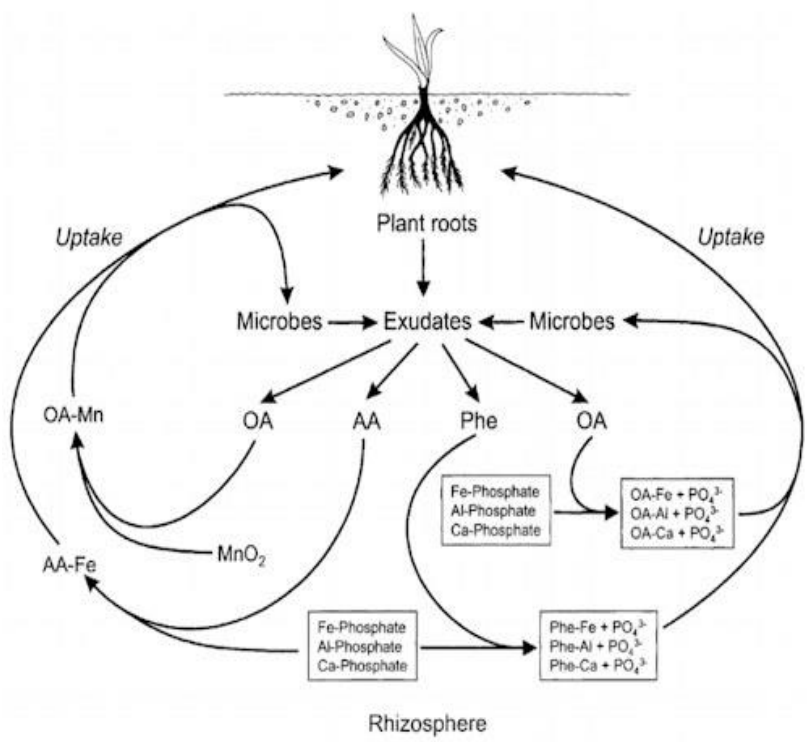

***Figure 1**. Rhizosphere interactions inducing nutrient solubilization and driving cycling system (Dakora et al, 2002)*

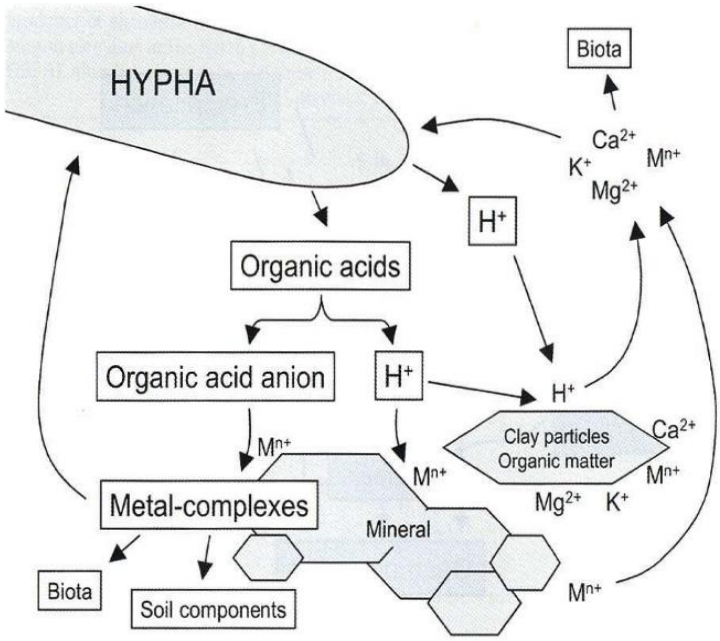

Figure 2. *Root exuded organic acids promoting nutrient exchange process (Gadd, 2004)*

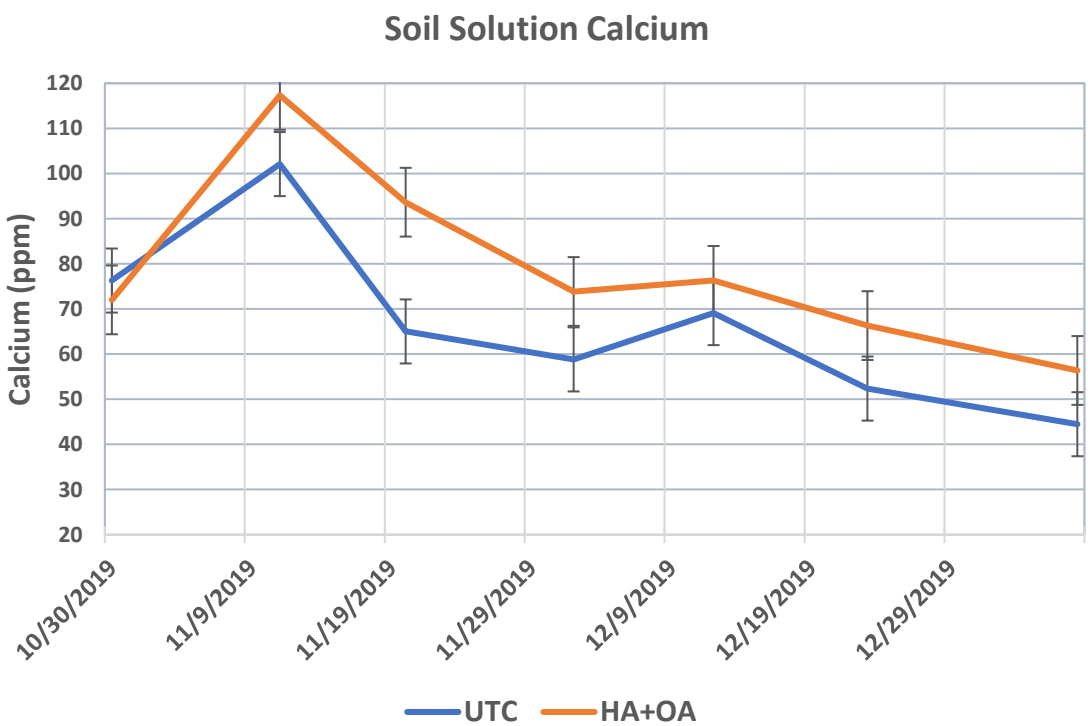

Figure 3. *Average calcium concentration in soil solution for the three study sites in Plant City, FL. Treatments include an untreated control (UTC) and humic acid + organic acid amended plots (HA+OA). Data were retrieved from 14 in-field lysimeters with the porous collection tip placed at a vertical depth of approximately 30cm from the top of the plastic mulch bed. Figure represents 7 collection dates through the early and middle portions of strawberry season (late October 2019 through early January 2020). Error bars represent standard errors of the means.*

***Table 2**: Comparison of fruit quality metrics between the control (UTC) and humic acid + organic acid amended plots (HA+OA). Averaged over 4 replications of 40ft linear sections harvested on 3 occasions (12/19/20, 01/23/20, and 02/19/20) for each treatment (p≤0.05).*

	UTC	HA + OA
Avg # Marketable Berries	*318.3 a*	*332.3 a*
Avg # Unmarketable Berries	*101.5 a*	*99.8 a*
Weight per Marketable Berry	*22.03g a*	*26.65g a*
BRIX	*7.1°Bx a*	*7.2°Bx a*
Fruit Firmness	*3.0kg/cm² a*	*3.0kg/cm² a*
Weight Loss After 9-Days in Storage (10-berries)	*42.5g a*	*41.5g a*
Calcium in Fruit-Tissue	*0.297% a*	*0.287% a*
Calcium in Leaf-Tissue	*1.11% a*	*1.15% a*

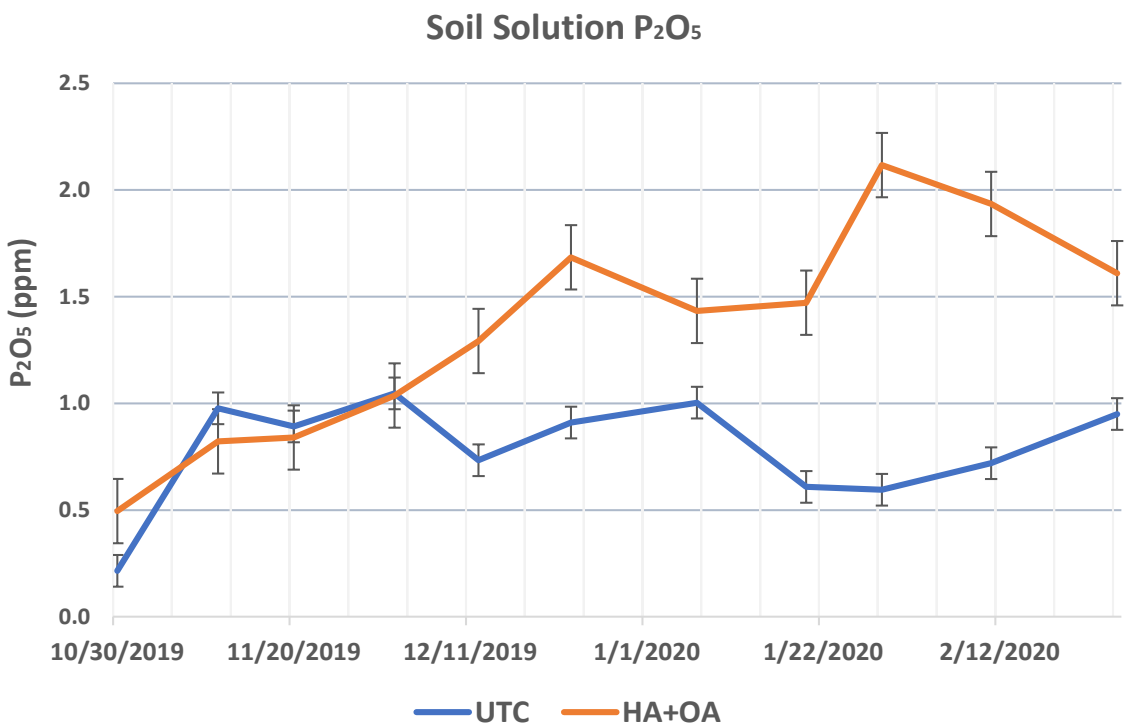

***Figure 4**. Average P_2O_5 concentration in soil solution for the three study sites in Plant City, FL. Treatments include an untreated control (UTC) and humic acid + organic acid amended plots (HA+OA). Data were retrieved from 14 in-field lysimeters with the porous collection tip placed at a vertical depth of approximately 30cm from the top of the plastic mulch bed. Figure represents 10 collection dates through the entirety of strawberry season (late October 2019 through late February 2020). Error bars represent standard errors of the means*

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Evaluating the Effects of Acetic Acid and d-Limonene on Four Aquatic Plants

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ADDITIONAL INDEX WORDS. **broadleaf sagittaria, citric acid, *Eichhornia crassipes*, invasive species, natural herbicides, organic herbicides, pickerelweed, *Pistia stratiotes*, *Pontederia cordata*, *Sagittaria latifolia*, vinegar, waterhyacinth, waterlettuce**

SUMMARY. **The foundation of most aquatic weed management programs in Florida is synthetic herbicides because many of these U.S. Environmental Protection Agency (USEPA)-registered products are effective, selective, and inexpensive compared with other strategies such as mechanical harvesting. However, stakeholders have expressed concern regarding their use and managers are interested in exploring alternative methods for aquatic weed control. To that end, we evaluated the efficacy, selectivity, and costs of the "natural" products acetic acid and d-limonene (alone and in combination with each other and citric acid) on the invasive floating plants waterhyacinth (*Eichhornia crassipes*) and waterlettuce (*Pistia stratiotes*), and the native emergent plants broadleaf sagittaria (*Sagittaria latifolia*) and pickerelweed (*Pontederia cordata*). These products, plus an industry-standard synthetic herbicide (diquat dibromide), were applied once as foliar treatments to healthy plants, which were grown out for 8 weeks after treatment to allow development of phytotoxicity symptoms. A 0.22% concentration of diquat dibromide eliminated all vegetation, but neither "natural" product alone provided acceptable (>80%) control of floating weeds, even when applied at the maximum concentrations under evaluation (20% acetic acid, 30% d-limonene). Citric acid (5% or 10%) had no effect on the activity of acetic acid or d-limonene, but some combinations of acetic acid and d-limonene controlled floating weeds effectively without causing unacceptable damage to native plants. However, these treatments are much more expensive than the synthetic standard and managers would realize a 22- to 26-fold increase in product cost alone without factoring in other expenses such as additional labor and application time. Combinations of acetic acid and d-limonene may have utility in some areas where the use of synthetic herbicides is discouraged, but broad-scale deployment of this strategy would likely be prohibitively expensive.**

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Florida's resource managers are charged with keeping aquatic vegetation at maintenance levels to facilitate navigation, flood control efforts, and other uses of state waters. This goal is most often achieved by using herbicides that have been approved by the U.S. Environmental Protection Agency (USEPA) for use in aquatic systems, with statewide oversight and coordination of treatments provided by the Florida Fish and Wildlife Conservation Commission (FWC, 2018, 2019a). For example, the FWC oversaw the expenditure of $17.007 million and $15.126 million in federal and state funds to control aquatic plants in Florida's public water bodies in fiscal year (FY) 2017–18 and FY 2018–19, respectively (FWC, 2018, 2019a). More than half of this funding ($10.01 million and $8.86 million in FY 2017–18 and FY 2018–19, respectively) was allocated for managing the submersed weed hydrilla (*Hydrilla verticillata*), whereas ≈25% of those monies ($4.04 million in FY 2017–18 and $4.19 million in FY 2018–19) was spent for floating plant control, which primarily comprise waterhyacinth (*Eichhornia crassipes*) and waterlettuce (*Pistia stratiotes*). Excessive growth of floating plants causes a number of problems in aquatic ecosystems, including reducing the penetration of oxygen and light into the water column by blocking the air–water interface, creating monocultures by outcompeting native plants, and interfering with flood control operations by creating large, dense mats that obstruct canals and water movement structures (Gettys, 2019). Waterhyacinth, a Brazilian species, was introduced intentionally to Florida during the late 1800s as a water garden ornamental and was released from cultivation soon thereafter (Gettys, 2020a). The native range of waterlettuce is cryptic and may include the southeastern United States, but the species exhibits aggressive growth and is considered invasive in Florida regardless of its true point of origin (Gettys, 2020b).

As with all pesticides registered by the USEPA, aquatic herbicides are only labeled for use if they "will not generally cause unreasonable adverse effects on the environment . . . taking into account the economic, social, and environmental costs and benefits of the use of any pesticide" (USEPA, 1996). However, public concerns regarding herbicide use in aquatic

Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
3.7854	gal	L	0.2642
9.3540	gal/acre	$L \cdot ha^{-1}$	0.1069
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
0.4536	lb	kg	2.2046
28.3495	oz	g	0.0353
1	ppm	$mg \cdot L^{-1}$	1
(°F – 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

systems have added to the challenges faced by managers. The FWC "paused" chemical weed management activities in early 2019 in response to public outcry and to provide an opportunity for stakeholder voices to be heard. A primary message that arose from listening sessions during the pause was that the public believes "chemical" (herbicide) usage in aquatic systems should be reduced drastically (FWC, 2019b). Although other aquatic weed management options such as mechanical harvesting do exist, most have greatly increased costs and reduced efficacy compared with chemical control tools. The need for "softer" products that can be used for aquatic weed control is urgent, and exploration is needed to identify ways for managers to maintain water resources effectively without the use of synthetic herbicides.

"Natural" herbicides are used extensively by home gardeners, organic farmers, and others who wish to reduce their use of synthetic herbicides. Products used for natural weed control include acids [i.e., acetic acid (vinegar) and citric acid], oils [clove (eugenol) (*Syzygium aromaticum*), pine (*Pinus* sp.), peppermint (*Mentha ×piperita*), and citronella (*Cymbopogon* sp.)], soaps, iron- or salt-based herbicides, corn (*Zea mays*) gluten, and combinations of these products (Smith-Fiola and Gill, 2017). Acids and oils may destroy cell membranes, which can lead to cell leakage, plant desiccation, and plant death (Baker, 1970; Webber et al., 2018). When used in upland (terrestrial) areas, these are nonselective contact foliar sprays that kill most broad-leaved weeds.

There are a number of commercially available natural herbicides that list acetic acid as the active ingredient. The most common acetic acid concentration in single-ingredient products is 20% [e.g., Maestro-Gro Organic Vinegar (Maestro-Gro, Justin, TX), Vinagreen Natural Non Selective Herbicide (Fleischmann's Vinegar Co., Cerritos, CA), Weed Pharm Fast Acting Weed and Grass Killer (Pharm Solutions, Port Townsend, WA)]. Products containing 20% acetic acid are typically labeled as ready to use (although some can be diluted to a concentration of 10%), and label instructions regarding spot treatments state that target weeds should be sprayed to wet. Products that include broadcast instructions indicate that 15 to 30 gal/acre of product should be used.

There are many other acetic acid products on the market, but most are not specifically labeled for weed control. For example, a note on the website for Bradfield Natural Horticultural Vinegar (20% acetic acid) states, "Although many folks, especially in the organic culture, have historically used strong vinegars to abate vegetation growth, be advised that Acetic Acids of 8% or less when characterized as an inert ingredient, in a mixture, are exempt from registration by the USEPA as a pesticide under USEPA 'Minimum Risk Pesticide' FIFRA 25B, List 4A. Thus this product (at 20% acidity) is not to be labeled, marketed or characterized in any way as having any herbicidal virtues" (Bradfield Industries, 2019; USEPA, 2004b). Horticultural vinegar, with an acetic acid concentration of 30%, is available, but is not listed specifically as a herbicide. Acetic acid is corrosive and can damage application equipment when used in highly concentrated form, but Evans et al. (2009) stated that lower concentrations can be used if application volume is increased to deliver the same total amount of acetic acid. For example, 15% acetic acid applied at 68 gal/acre will reportedly yield similar results as 30% acetic acid at 34 gal/acre (Quarles, 2010).

Although citric acid is often a component in "natural" herbicide mixes, products that rely only on citric acid or citrus oil are less common. Avenger Weed Killer Concentrate (Cutting Edge Formulations, Buford, GA) contains 70% d-limonene. The label indicates the product should be diluted at 1:6 (10% d-limonene for small annual weeds) to 1:3 (17.5% d-limonene) for hard-to-control weeds, then sprayed to wet. GreenMatch Burndown Herbicide (Cutting Edge Formulations) is 55% d-limonene, and label instructions specify diluting at a 1:6 ratio (8% d-limonene) for broadcast treatments or a 1:4 ratio (11% d-limonene) for spot treatments.

Despite widespread interest in reducing synthetic herbicide use, there is a dearth of information available in the scientific literature regarding the efficacy of natural products such as acetic acid and d-limonene as weed control agents, either alone or in combination. Domenghini (2020) reported that acetic acid applied at a concentration of 20% or 30% could be a viable alternative to glyphosate, but that multiple applications would be necessary for prolonged weed control. Webber et al. (2018) evaluated 5% and 20% acetic acid solutions alone or with sweet orange (*Citrus sinensis*) or canola (*Brassica napus*) oil; they found that weed control increased as acetic acid concentration increased and that there was little or no advantage to adding either type of oil to the acetic acid solutions. Evans and Bellinder (2009) stated that broadcast applications of 15%, 20%, and 30% acetic acid mixed with 1.7% or 3.4% clove oil could be useful for weed suppression in sweet corn, onion (*Allium cepa*), and potato (*Solanum tuberosum*) cultivation. Shrestha et al. (2012) reported that a single application of 20% d-limonene provided up to 95% weed control in organic almond (*Prunus dulcis*) orchards 1 week after treatment, but that efficacy was reduced to 53% control 5 weeks after treatment, necessitating repeat applications every 5 to 6 weeks.

Even less information is available in the scientific literature regarding the use of natural products for weed control in aquatic ecosystems. These products are not labeled for use as herbicides in aquatic areas, so they have not been subjected to the many tests required before USEPA approval, which include environmental fate and ecological toxicity assessments (Stubbs and Layne, 2020), and their effects on aquatic fauna have not been well-characterized. However, Saha et al. (2006) reported that acetic acid had a 96-h 50% lethal concentration value of 273 $mg \cdot L^{-1}$ on tilapia (*Oreochromis mossambicus*) (i.e., tilapia populations exposed to this concentration for 96 h would be expected to experience death of half the population), and that dissolved oxygen and plankton populations were reduced after exposure to 17 $mg \cdot L^{-1}$ acetic acid. The USEPA (2004a) stated that technical-grade and formulated d-limonene is "practically nontoxic or slightly toxic to birds, fish and invertebrates," but Kim et al. (2013) reported that

metabolites of d-limonene may cause skin irritation in humans and other animals. As stated earlier, acids and oils can destroy cell membranes (Baker, 1970; Webber et al., 2018) and thus should be considered nonselective and likely to cause damage to off-target flora that comes into contact with these products. Anderson (2007) reported that rhizomes of the emergent aquatic weed smooth cordgrass (*Spartina alterniflora*) had 90% reductions in shoot number and plant height 9 months after exposure to acetic acid. Acetic acid reduces hydrilla regrowth from root crowns (Spencer and Ksander, 1995), and inhibits viability and sprouting of hydrilla and sago pondweed (*Stuckenia pectinata*) tubers (Spencer and Ksander, 1997, 1999). However, using acetic acid to suppress submersed weed growth would only be practical when employed in conjunction with dewatering, because achieving an adequate concentration of acetic acid in the entire water column is virtually impossible and could cause significant off-target damage to other flora and fauna in the system. For these reasons, the scope of this research is limited to testing the effects of contact products on above-water vegetation (i.e., floating and emergent plant material).

The primary goals of this project were to evaluate the effects of acetic acid and d-limonene (alone and in combination with each other) on two floating invasive target species and two emergent desirable nontarget species, and to compare the costs of using these products vs. a synthetic USEPA-approved aquatic herbicide. According to the FWC National Pollution Discharge Elimination System report for calendar year 2018, diquat dibromide, which is nonselective, was the most commonly used herbicide for floating weed management, with a total of 7871.77 gal of formulation (37.3% diquat dibromide) applied (Clark and Dew, 2019), so this product will serve as the synthetic "standard practice" treatment in these experiments.

Materials and methods

Efficacy studies. Target (weed) species were waterhyacinth and waterlettuce, whereas nontarget (desirable) species were pickerelweed (*Pontederia cordata*) and broadleaf sagittaria (*Sagittaria latifolia*). Plants were treated in pairs of one invasive floating species and one native emergent species. Run 1 focused on waterhyacinth and broadleaf sagittaria, whereas run 2 focused on waterlettuce and pickerelweed.

Target species were field-collected or pulled from cultures maintained at the University of Florida Fort Lauderdale Research and Education Center (FLREC) in Davie, FL, and were moved to 18-gal plastic tubs filled with well water. Run 1 tubs (waterhyacinth) were amended with 10 g each of crushed 15N–3.9P–10K controlled-release fertilizer formulated for 6-month release in Florida (Osmocote Plus; ICL Specialty Fertilizers, Dublin, OH) and 7N–0P–0K iron chelate micronutrient (Sprint 330; BASF Corp., Research Triangle Park, NC). Run 2 tubs (waterlettuce) were amended with 3.4 g of 24N–3.5P–13.3K water-soluble fertilizer (Miracle-Gro Water Soluble All Purpose Plant Food; Scotts Miracle-Gro Products, Marysville, OH) and 1.2 g of 7N–0P–0K iron chelate micronutrient. Each tub was initially "seeded" with 10 plants of a target species, which were grown out for 4 to 6 weeks to allow development of more than 80% surface coverage.

Nontarget species were purchased from an aquatic nursery (Aquatic Plants of Florida, Myakka City, FL) and transported to a greenhouse at FLREC, where individual plants were transplanted into 2-L plastic pots without holes that were filled with sand [grain diameter 0.25–0.5 mm (Multi-Purpose Sand; Sakrete, Charlotte, NC)] amended with 4 g of the same controlled-release fertilizer used in run 1 tubs. Plants were grown out on greenhouse benches and irrigated twice per day (10:00 AM and 4:00 PM) with the equivalent of 0.5 inch of water per irrigation before being used in experiments. New shoots were cut back during this culture period to ensure that each 2-L pot contained a single nontarget plant. When target plant coverage reached more than 80% of the surface of the water, one potted nontarget plant was introduced to each tub (water depth, ≈20 cm above the surface of the pots) and all plants were then subjected to treatment.

Treatments were applied as single spot "spray to wet" foliar applications to above-water foliage, and all treatments included 1% v/v of a nonionic surfactant (Induce; Helena Agri-Enterprises, Collierville, TN) to aid in penetration and emulsification. Nine single-product treatments (5%, 7.5%, 10%, 15%, and 20% acetic acid; and 10%, 15%, 20%, and 30% d-limonene), 30 combination treatments (all combinations of single acetic acid and d-limonene treatments; all concentrations of acetic acid plus 5% and 10% citric acid), three synthetic standard-practice treatments (0.22%, 0.45%, and 0.89% diquat dibromide), and an untreated control were evaluated, with four replicates of each treatment. Base materials were 30% acetic acid (Green Gobbler 30% Vinegar Home and Garden; EcoClean Solutions, Copiague, NY), 100% d-limonene (100% Pure Technical Grade D-Limonene, EcoClean Solutions), 37.3% diquat dibromide (Tribune Herbicide; Syngenta Crop Protection, Greensboro, NC), and 100% citric acid (Milliard Citric Acid; Milliard Brands, Lakewood, NJ). Treatments were applied to run 1 and run 2 plants on 12 Nov. 2019 and 15 Jan. 2020, respectively. Plants were monitored weekly for 8 weeks after treatment and then the project lead assigned a numerical value of 0 through 10 to describe visual quality (0 = dead; 5 = fair quality, acceptable, somewhat desirable form and color, little to no chlorosis or necrosis; 10 = excellent quality, perfect condition, healthy and robust, excellent color and form). Although some authors (e.g., Cutelle et al., 2013; Koschnick et al., 2005; Mudge et al., 2007) report visual injury or damage resulting from herbicide treatments, we recorded visual quality, which has also been used to describe plant response to differing culture conditions (e.g., Gettys and Moore, 2018, 2019), herbicides (e.g., Gettys and Haller, 2009, 2010, 2012; Smith et al., 2014), salt stress (e.g., Tootoonchi et al., 2020), and other experimental factors. After visual scoring, a destructive harvest was conducted to collect all live biomass of floating species and all live aboveground shoots of emergent species. Harvested materials were placed

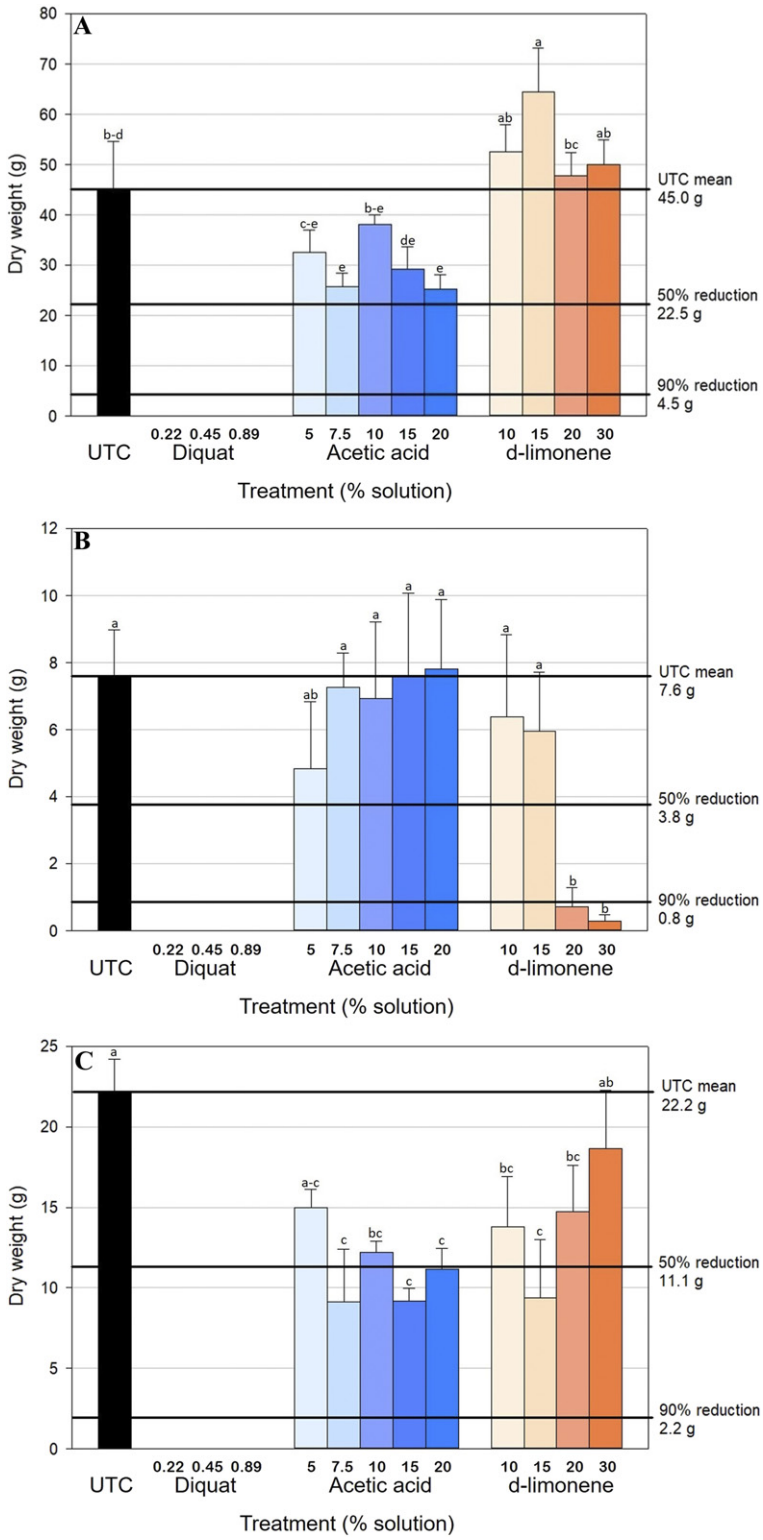

Fig. 1. Biomass of (A) waterhyacinth, (B) waterlettuce, and (C) pickerelweed 8 weeks after single-product treatment. Bars are the mean of four replicates and error bars represent 1 SD from the mean. Treatments coded with the same letter are not different at $P = 0.05$. The upper bold horizontal rule indicates the mean of untreated control (UTC) plants, whereas the central and lower bold horizontal rules indicate 50% and 90% reductions compared with UTC plants; 1 g = 0.0353 oz.

in paper bags and moved to a forced-air oven maintained at 65 °C for 2 weeks before being weighed. Visual evaluations and destructive harvests occurred on 7–9 Jan. 2020 (run 1) and 11–13 Mar. 2020 (run 2).

Visual data were arcsine-transformed before analysis to normalize distribution. Data within each species were evaluated using a generalized linear model (SAS version 9.4; SAS Institute, Cary, NC) to determine whether treatment means differed from those of untreated plants at $P = 0.05$. Treatment means of visual values and dried biomass were then compared with untreated controls. Haller and Gettys (2013) reported that an ideal herbicide treatment should cause a >90% reduction in these parameters in target weeds and a <50% reduction in nontarget native plants. Therefore, we used these values as benchmarks for efficacy on the floating weeds waterhyacinth and waterlettuce, and selectivity on the emergent native plants pickerelweed and broadleaf sagittaria.

Cost comparisons. A total of four diquat dibromide products, all formulated as 37.3% diquat dibromide, were used by the FWC in FY 2018–19 [67 gal Alligare Diquat (Alligare, Opelika, AL), 368 gal Diquat SPC 2L (Nufarm Americas, Burr Ridge, IL), 34 gal Reward (Syngenta Crop Protection), and 7407.77 gal Tribune (Syngenta Crop Protection)]. The majority (>94%) of diquat dibromide was applied as Tribune, which was purchased at the FWC's negotiated contract price of $35.50/gal (vendor, Helena Agri-Enterprises; size, 2.5-gal jug) (Clark and Dew, 2019; Cleary and McNiel, 2019). As such, cost comparisons will assume a purchase price of $35.50/gal for all diquat dibromide products.

Small volumes (e.g., 1 gal) of 30% acetic acid and technical grade d-limonene are $24.99/gal and $59.99/gal, respectively (Factory Direct Chemicals, 2019a, 2019b). When purchased in bulk, 30% acetic acid is $8.00/gal (275-gal tote) and technical grade d-limonene is $31.82/gal (4 × 55-gal drums) (Factory Direct Chemicals, 2019a, 2019b). If acetic acid and d-limonene were to be used for aquatic weed control in Florida, it is likely they would be purchased in bulk, so cost comparisons will assume a purchase price of $8.00/gal for 30% acetic acid and $31.82/gal for technical grade d-limonene.

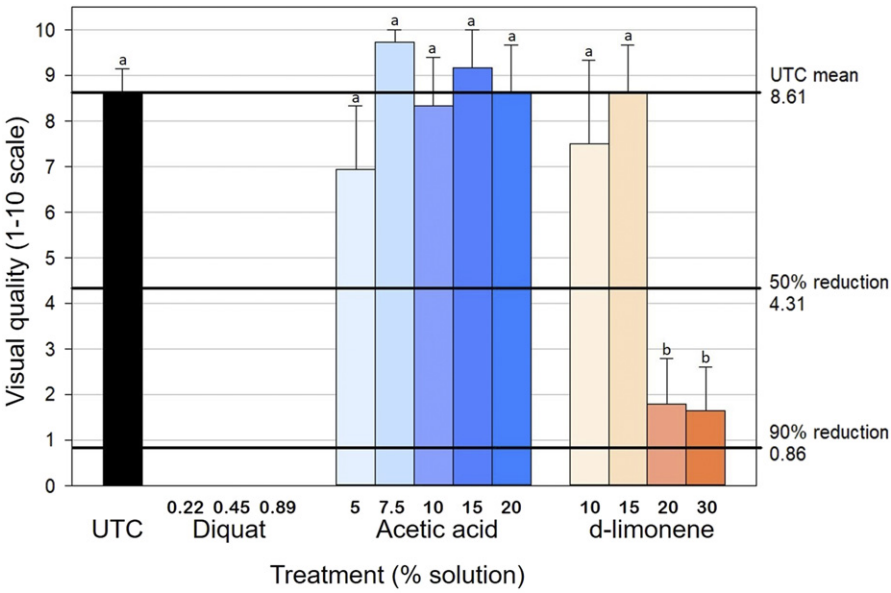

Fig. 2. Visual quality of waterlettuce 8 weeks after single-product treatment. A numerical scale of 0 through 10 is used to describe visual quality, where 0 is dead; 5 is fair quality, acceptable, somewhat desirable form and color, little to no chlorosis or necrosis; and 10 is excellent quality, perfect condition, healthy and robust, excellent color and form. Bars are the mean of four replicates and error bars represent 1 SD from the mean. Treatments coded with the same letter are not different at $P = 0.05$. The upper bold horizontal rule indicates the mean of untreated control (UTC) plants, whereas the central and lower bold horizontal rules indicate 50% and 90% reductions compared with UTC plants.

Results

Single products. The only single-product treatments that provided good control of both waterhyacinth and waterlettuce were diquat dibromide at 0.22%, 0.45%, and 0.89%, with all three concentrations completely eliminating both floating weeds. Unfortunately, all nontarget native plants were eliminated by these treatments as well. Although a goal of these experiments was to compare the efficacy of natural products to the synthetic herbicide diquat dibromide, it became clear that most natural treatments were much less effective than diquat dibromide, and comparisons between natural treatments and untreated controls would be more informative. Thus, diquat dibromide treatments were removed from data sets before further statistical analyses were conducted. Waterhyacinth biomass was affected by single-product natural treatments [$P < 0.01$ (Fig. 1A)], but no treatment reduced biomass by >50% or affected visual quality ($P = 0.66$). These treatments had an effect on waterlettuce biomass [$P = 0.03$ (Fig. 1B)] and visual quality [$P < 0.01$ (Fig. 2)], which were reduced by >90% and >75%, respectively, after treatment with 20% or 30% d-limonene, but no other single-product treatments were different from untreated control plants. Pickerelweed dry weight was reduced by most single-product treatments compared with untreated control plants [$P = 0.01$ (Fig. 1C)], but none reduced weight by >80%, and visual quality was unaffected ($P = 0.16$). Single natural products had no effect on broadleaf sagittaria dry weight ($P = 0.48$) or visual quality ($P = 0.68$).

Acetic acid and citric acid mixes. Combinations of 5% to 20% acetic acid and 5% or 10% citric acid did not reduce biomass or visual quality by at least 50% in any of the species evaluated in these experiments. Dry weight and visual quality of treated and untreated plants were not different ($P = 0.06$ to $P = 0.89$) in most cases. The sole exception was dry weight of waterlettuce ($P = 0.02$); treated plants were not different from untreated plants, but differences were detected among treatment combinations.

Acetic acid and d-limonene mixes. In contrast to single-product natural herbicide treatments and mixes of acetic and citric acids, some combinations of acetic acid and d-limonene had good efficacy on both floating weeds (waterhyacinth and waterlettuce biomass and visual quality, $P < 0.01$). The most promising combinations on waterhyacinth were 15% acetic acid plus 15%, 20%, or 30% d-limonene and 20% acetic acid with any concentration of d-limonene; these treatments reduced dry biomass by >80% (Fig. 3A) and visual quality by >60% (Fig. 4A). Most combinations of acetic acid and d-limonene had good efficacy on waterlettuce; dry weight (Fig. 3B) and visual quality ratings (Fig. 4B) were reduced compared with untreated control plants in all but a single treatment (5% acetic acid plus 10% d-limonene). Only 5 of the 20 treatment combinations failed to reduce biomass by >90% compared with untreated control plants, and two combinations failed to reduce visual quality by >50%. Pickerelweed biomass and visual quality were affected by combinations of acetic acid and d-limonene. Biomass was reduced in plants treated with any combination of acetic acid and d-limonene compared with untreated control plants [$P < 0.01$ (Fig. 3C)], and visual quality was reduced in 13 of the 20 treatments [$P = 0.03$ (Fig. 4C)]. Broadleaf sagittaria biomass was unaffected by mixes of acetic acid and d-limonene ($P = 0.34$). Visual quality of sagittaria was affected [$P = 0.04$ (Fig. 4D)], but treated plants were not different from untreated plants and all differences occurred among treatment combinations.

These results suggest that some combination treatments of acetic acid and d-limonene may be useful for managing populations of invasive waterhyacinth and waterlettuce while providing a level of selectivity with reduced damage to the native plants broadleaf sagittaria and pickerelweed.

Cost analysis of acetic acid and d-limonene mixes for floating weed management. The synthetic standard-practice treatments in these experiments used diquat dibromide. These experiments evaluated foliar treatments only and we envision that field treatments for floating weed management would be applied as spot treatments as opposed to broadcast treatments. Diquat dibromide can be applied at a concentration of up to 2% formulated product (equivalent to 0.89% diquat dibromide) for floating

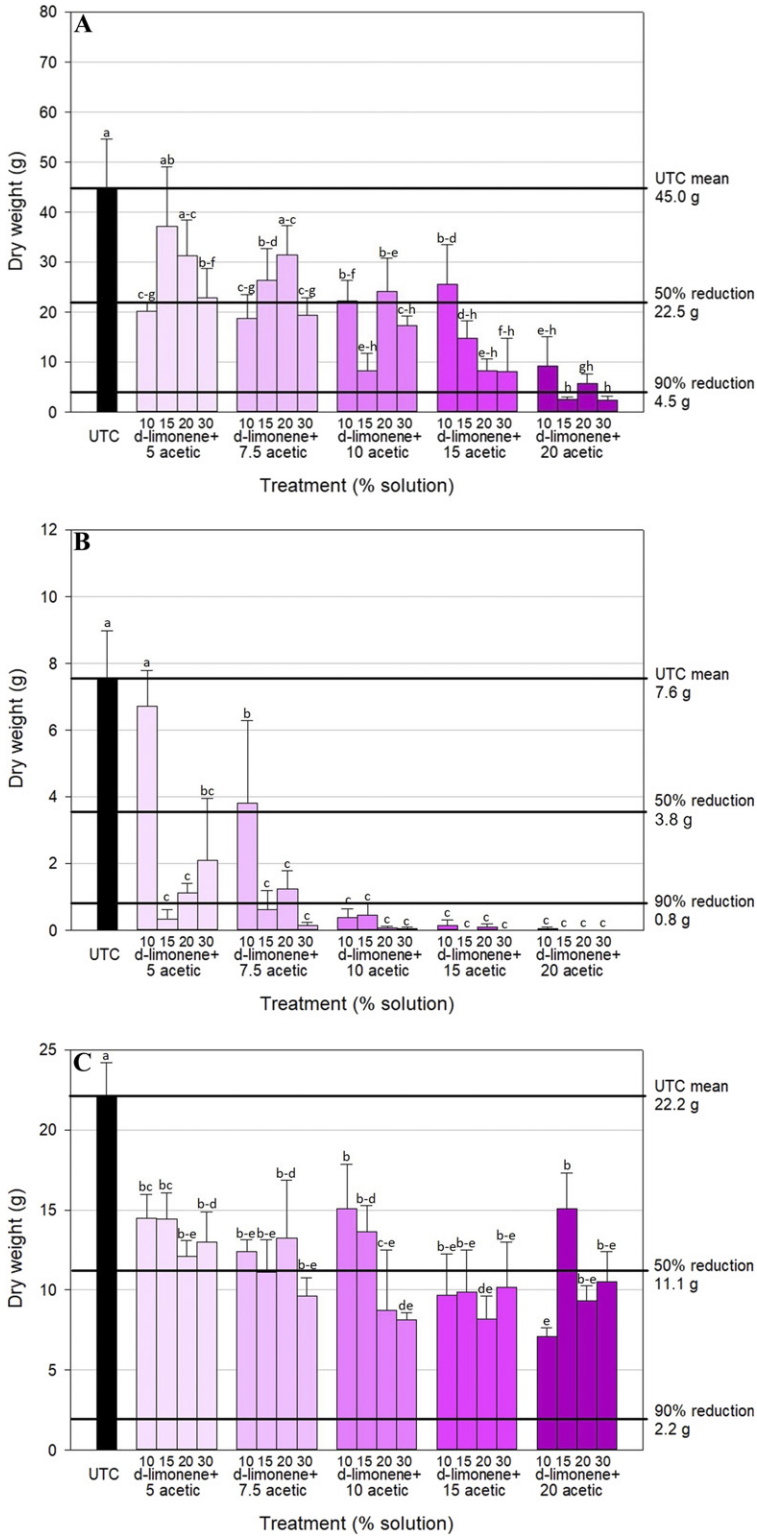

Fig. 3. Biomass of (A) waterhyacinth, (B) waterlettuce, and (C) pickerelweed 8 weeks after treatment with combinations of acetic acid and d-limonene. Bars are the mean of four replicates and error bars are 1 SD from the mean. Treatments coded with the same letter are not different at $P = 0.05$. The upper bold horizontal rule is the mean of untreated control (UTC) plants, whereas the central and lower bold horizontal rules indicate 50% and 90% reductions compared with UTC plants; 1 g = 0.0353 oz.

and marginal weeds, whereas spot treatments should use a concentration of 0.5% formulated product (equivalent to 0.22% diquat dibromide) (Syngenta Crop Protection, 2011). We evaluated three concentrations (0.22%, 0.45%, and 0.89%) of diquat dibromide, but the lowest concentration completely eliminated all plant material, so calculations are based on spot treatments using a 0.22% solution. As mentioned in the Materials and Methods, the FWC applied a total of 7871.77 gal of 37.3% diquat dibromide to floating plants in 2018 (Clark and Dew, 2019). Assuming all diquat field treatments were mixed to a concentration of 0.22% diquat dibromide, a total of 1,574,354 gal of ready-to-use (RTU) mix was made from the 7871.77 gal of concentrate purchased. The FWC's contract price for 37.3% diquat dibromide was \$35.50/gal, resulting in a total cost of \$283,383.72; after dilution to a 0.22% concentration, the final cost is \$0.1775/gal RTU, or ≈\$0.18/gal RTU.

As mentioned in the Materials and Methods, these calculations are based on purchase prices of \$8.00/gal for 30% acetic acid and \$31.82/gal for technical grade d-limonene. Neither acetic acid nor d-limonene resulted in acceptable control of waterhyacinth when applied alone, but some combinations of the two provided good control (>80% reduction in biomass) of waterhyacinth. These were 15% acetic acid plus 15%, 20%, or 30% d-limonene and 20% acetic acid with any concentration of d-limonene. The material costs to make RTU 15% or 20% acetic acid are \$1.20/gal or \$1.60/gal, respectively. The material costs for RTU d-limonene are \$3.18/gal (10%), \$4.77/gal (15%), \$6.36/gal (20%), and \$9.55/gal (30%). Thus, the least expensive efficacious natural treatment (20% acetic acid + 10% d-limonene; \$1.60 + \$3.18) for waterhyacinth is \$4.78/gal RTU, or nearly 26× more expensive than the synthetic standard (0.22% diquat dibromide) treatment (\$0.18/gal RTU). Assuming other application parameters (e.g., surfactant cost, labor) remain unchanged between the treatment types, and remembering that a total of 1,574,354 gal of RTU mix was used in 2018, switching completely from synthetic to natural products for waterhyacinth management would increase single-year product costs from \$283,383.72 (0.22% diquat dibromide) to \$7,525,412.12 (20% acetic acid + 10% d-limonene).

In contrast to waterhyacinth, there were many treatments that reduced waterlettuce biomass by at least 90% compared with untreated control

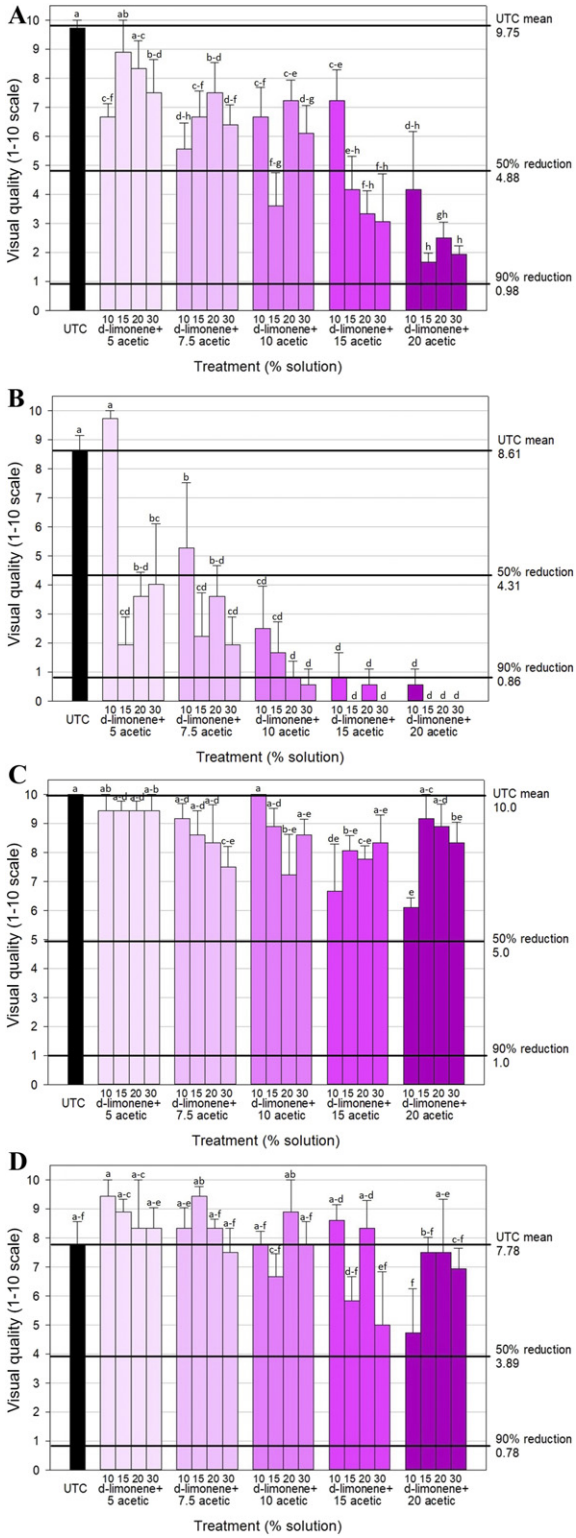

Fig. 4. Visual quality of (A) waterhyacinth, (B) waterlettuce, (C) pickerelweed, and (D) broadleaf sagittaria 8 weeks after treatment with combinations of acetic acid and d-limonene. A numerical scale of 0 through 10 is used to describe visual quality, where 0 is dead; 5 is fair quality, acceptable, somewhat desirable form and color, little to no chlorosis or necrosis; and 10 is excellent quality, perfect condition, healthy and robust, excellent color and form. Bars are the mean of four replicates and error bars represent 1 SD from the mean. Treatments coded with the same letter are not different at $P = 0.05$. The upper bold horizontal rule indicates the mean of untreated control (UTC) plants, whereas the central and lower bold horizontal rules indicate 50% and 90% reductions compared with UTC plants.

plants. Most efficacious treatments used combinations of acetic acid and d-limonene, but applications of 20% and 30% d-limonene alone also reduced waterlettuce biomass by 90%. The least expensive efficacious natural treatment (10% acetic acid + 10% d-limonene; \$0.80 + \$3.18) for waterlettuce is \$3.98/gal RTU, or ≈22× more expensive than the synthetic standard treatment (\$0.18/gal RTU). As with the caveats just described for waterhyacinth, switching completely from synthetic to natural products for waterlettuce management would increase single-year product costs from \$283,383.72 (0.22% diquat dibromide) to \$6,265,928.92 (10% acetic acid + 10% d-limonene).

The figures calculated here do not account for the likelihood that applications using natural products would take longer, resulting from the need to transport very large volumes of base material. For example, consider a spray boat with a 100-gal tank that uses diluent water drawn from the system being treated. Filling the tank once for a synthetic treatment would require the transport of 0.5 gal of 37.3% diquat dibromide, but filling the tank once with the least expensive efficacious natural treatment for waterhyacinth management would require the transport of 67 gal of 30% acetic acid and 10 gal of technical grade d-limonene, or ≈630 lb of materials (without factoring in the weight of the containers used to transport the materials). Rather than transporting base materials and adding them to the tank at the treatment site, applicators would likely add the natural treatment components to the tank at the ramp and would thus have to return to shore for reloading after applying 100 gal of RTU natural mix. In contrast, an applicator with a single 2.5-gal jug of 37.3% diquat dibromide would have enough base material to make 500 gal of RTU synthetic mix without returning to the ramp. As a result, replacing synthetic herbicides with natural products would not only greatly increase material costs but would also decrease productivity (as measured in acres treated per day).

Discussion

The natural products evaluated in these studies may have some utility for managing floating weeds such as waterhyacinth and waterlettuce selectively without causing unacceptable levels of

damage to desirable native plants such as broadleaf sagittaria and pickerelweed. Acetic acid alone did not cause adequate (>50% reduction in biomass and visual quality) damage to either weed species, whereas d-limonene alone caused >90% reduction in biomass of waterlettuce only when applied at concentrations ≥20%. The addition of 5% or 10% citric acid to acetic acid had no effect on acetic acid efficacy, so further investigations with citric acid are not recommended. Some combinations of acetic acid and d-limonene provided acceptable control of both floating weeds, with biomass reductions of ≥80%. Waterlettuce was more sensitive to treatments than was waterhyacinth, which is likely a result of the structure of the plants. Both floating weeds have a rosette form, with leaves attaching to the base of the plant. However, the sessile leaves of waterlettuce create a "bowl" that can capture and hold liquids, which can result in longer exposure times, whereas the petiolate leaves of waterhyacinth facilitate drainage of liquids through the plant and into the water column.

Regardless of the target species and selected treatment chosen, replacement of current industry-standard aquatic herbicides with these natural products would result in profound increases in management costs. As mentioned, the material-only cost of treating floating weeds in Florida with 0.22% diquat dibromide in 2018 was $283,383.72. Treating a similar area with natural products would increase material-only costs to $7,525,412.12 for waterhyacinth and $6,265,928.92 for waterlettuce. Therefore, these natural products may have utility in select areas where the use of synthetic herbicides is discouraged, but broad-scale deployment of this management strategy would likely be prohibitively expensive.

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Martin County, Florida Food System Study[1]

William A. Messina, Jr., Lisa House, Yvette Goodiel, and Carol Alberts[2]

Background

Martin County has a rich agricultural history with farming (particularly citrus) and cattle ranching being important economic drivers since the early 1930s (Martin County Business Development Board). Even today, almost 50 percent of the county's land area remains in agricultural production (Martin County Property Appraiser 2019).

According to the most recent (2017) federal Census of Agriculture, Martin County has 594 farms, with 153,732 acres of agricultural land (USDA-NASS 2019). A study by the UF/IFAS Food and Resource Economics Department calculates that in 2016, agriculture, natural resources, and related food industries accounted for 24.2 percent of employment in Martin County, and $1.2 billion worth of Value Added Contribution,[1] which represents over 18 percent of the county's total Value Added Contribution (Court, Hodges, and Rahmani 2018).

Food crops produced in Martin County include vegetables, melons, fruits, sugarcane, fish, poultry/eggs, and beef. The principal vegetable and melon crops grown in Martin County include cabbages, potatoes, tomatoes, and watermelons (FDACS 2016). Top commercial fishing landings are of Spanish and King mackerel (Butch Olsen, Port Salerno Commercial Fishing Dock Authority, personal communication). Court, Hodges, and Rahmani (2018) estimate value added contributions of $8M for vegetable and melon farming, $61M for fruit farming, $23M for commercial fishing, $6M for poultry and egg production, and $7M for beef cattle ranching and farming.

While agricultural production occurs on almost half of the county's land and accounts for almost 20 percent of the county's total value added contribution, the Martin County region struggles to meet the balance between food supply and demand. Farmers often struggle to find sizable, secure, well-paying markets, and the vast majority of consumers do not participate in local food transactions. It therefore was anticipated that the food supply chain in the region might benefit from a "food hub," defined by the USDA as a "centrally located facility with a business management structure that facilitates the aggregation, storage, processing, distribution, and/or marketing of locally/regionally produced food products" (USDA-AMS 2010: 1).

The Martin County Food System Feasibility Study, funded by a USDA Rural Business Development Grant, identified regional food system constraints and opportunities, including opportunities for a food hub and/or farm incubator to strengthen the food supply chain and opportunities for expanding connectivity among supply chain participants to improve food security and increase access to local foods. While focus was on a county and its surrounding areas, the

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This publication is a summary of two longer reports: Martin County Food System Feasibility Study and Martin County Food System Infrastructure Analysis.

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Martin County study methodology can serve as a template for similar studies in other regions, and the study results can contribute toward a better understanding of food system constraints and opportunities on a broader level.

Methods

The study incorporated over a dozen interviews of supply side participants in the Martin County food system (e.g., growers and producers), and hundreds of demand side interviews (e.g., with consumers, restaurants, food distributors, the local school system, etc.), to assess current food system needs, as well as to analyze existing infrastructure and its potential to address constraints and support opportunities for food system improvement. Local stakeholders serving on the Martin County Agriculture and Natural Resources Advisory Committee helped to guide efforts during the study, developing a list of potential interviewees and reviewing draft survey tools. Additionally, representatives from the Martin County Business Development Board, Treasure Coast Regional Planning Council, Martin County Economic Development Council, and the Martin County Growth Management Department provided input on the draft reports, particularly in terms of potential food system resources, constraints, and opportunities.

Supply side interviews were conducted with local growers to better understand their current operations and local consumer marketing efforts and to gauge their interest in potential food system enhancements, such as a food hub, expanded local food supply for low-income residents, and/or a farm incubator program.

Demand side interviewees included wholesale distributors, large institutional buyers, retail produce markets, restaurants, and approximately 350 Martin County residents. Interviewees provided information on their purchases of fruit/vegetable/protein sources, including pricing, purchase criteria, desire for "local" foods, types of fresh fruits/vegetables/proteins purchased, purchase locations, transportation, and constraints to purchasing fresh produce and proteins. Additional input was provided by consumers and agribusinesses through public meetings and online surveys.

The analysis of existing food system infrastructure was conducted through interviews with operators of existing infrastructure facilities. Interviewees were asked to identify the capabilities of their operation (processing, packing, commercial kitchen, storage, etc.), discuss sourcing and transport of products, share information on the types of buyers served, note primary months of operation, and indicate whether there would be capacity for additional products. Lastly, interviewees were asked to share their perspective on the primary needs of the local food system.

Recommendations

Through the interviews and surveys, it became clear that the region has a strong, though somewhat fragmented, network of growers, distributors, food pantries/banks, small-scale food hubs, processing facilities, markets, and commercial kitchens. Demand side operations expressed a need for additional supply, and over 90 percent of consumer respondents indicated that they think "buying local" is very or at least somewhat important. Therefore, an opportunity exists in Martin County to make the most of the existing food chain structures and non-profit participants and build upon them to potentially cultivate new producers and market channels to better meet local demand. Study results and recommendations have been shared with local decision-makers and other stakeholders to inform future policies and programs.

In determining how to make the best use of existing infrastructure, several key components are recommended, including better connecting participants along the food chain to maximize efficiencies; examining approaches other communities have taken to improve their local food systems; engaging agribusiness and food entrepreneurs in productive discussions with local policy-makers to clarify and revise regulations where needed; training food chain staff and volunteers in food production, food safety, and other needed skill sets; and offering branding options to help producers better market their produce to local consumers. The experience and knowledge of growers who supply local markets, as well as those who supply a wider distribution network, can inform efforts to maximize use of existing local food infrastructure. For example, when asked about the feasibility of supplying a food hub outlet in the region, growers shared that pricing and transportation would first need to be addressed. Additionally, the risk of shifting from established relationships with buyers and distributors to a new hub might preclude growers from participating, unless they could begin engaging through provision of excess product. By more closely connecting growers with one another and with demand side buyers, all could learn more of each other's capabilities, share food processing resources, improve outreach to and accessibility for consumers, and communicate needs or excess supply year-round.

As food chain participants engage with one another locally, they can also learn from food system innovations in other communities, including mobile food pantries and food

hubs; hearing from communities about not only their successes but also their failures can provide valuable insights. Where local food system developments are stymied by concerns over regulations, dialogue with policy-makers and regulators can help to clarify regulations, thereby reducing risk and uncertainty. Where needed, dialogue can lead to policy change, particularly where regulations may not be adapted to, or comprehensive of, new food system innovations.

Risk and uncertainty can also be addressed through training of food system staff and volunteers; interviewees identified training needs in areas such as food safety, food production, methods or systems to allow extension of the production season, alternative crops, hospitality, and worker safety.

Marketing and branding were areas where some felt that additional education and resources would be of benefit. As a first step, connecting more food chain participants (growers, restaurants, farmers markets, wholesalers, distributors, pantries, etc.) with the Florida Department of Agriculture and Consumer Services promotional campaign's "Fresh from Florida" branding services could help businesses and non-profits capitalize on an established state-wide logo and marketing effort in advertising their products to consumers. Much can be accomplished for the regional food system by drawing on the strengths of local food industry leaders and innovators; connecting them with one another, with local policy-makers, and with food system innovators in other regions; and addressing needs for training, marketing, and other resources.

Given the demand for additional local product, new producers are another vital component for the region's food system. The idea of a farm incubator or a network of small incubators, which would provide training and technical assistance for beginning farmers, was greeted with considerable interest by small farmers, market managers, and others. Participants saw existing community gardens as forms of pilot incubators, capable of sharing information on costs, by-laws, infrastructure needs, etc. to guide start-up. Potential partners identified by stakeholders include the Boys and Girls Club, Farm Bureau's Beginning Farmer and Rancher Program, South Fork High School, UF/IFAS Extension, and local farmers. Before an incubator could be established, a lead agency, organization, or business would need to commit to the project; a group of motivated and dedicated participants would need to be identified; and start-up resources (land, funding, etc.) would need to be secured. Lessons learned and materials developed could then be shared with other stakeholders interested in developing new incubators.

The local food system benefits the Martin County region through provision of fresh produce and protein sources, employment opportunities, and value added economic contributions. With strong demand for local food and a diverse network of growers, food entrepreneurs, food pantries, and markets, the region has an opportunity to more strongly connect with and support existing food system participants. Strengthening the existing food system network can build a path for new producers to enter the marketplace more readily, contributing further to the local economy, improving food security, and expanding access to local foods.

Note

[1] Value Added Contribution is a broad measure of income, representing the sum of employee compensation, proprietor income, other property income, indirect business taxes and capital consumption (depreciation). Value added is a commonly used measure of the contribution of an industry to a regional economy because it avoids double counting of intermediate sales (Court, Hodges and Rahmani 2018).

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Increasing Adoption of Water-saving Technologies on Farms

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As an effort to improve water use efficiency in Alachua County's horticultural crops, the agent started an on-farm program in 2018 to demonstrate the use and benefits of installing soil moisture sensors (SMS). The goals were to increase awareness of Best Management Practices (BMPs) and adoption of water-saving technologies and practices to improve water use in farms. Resources to conduct the demos using All-in-one Sentek Soil Moisture Probeswere acquired from the University of Florida/IFAS Minigrant program and the Northwest Florida Research and Education Center-Suwannee Valley.

The probes were rotated on six farms with a variety of vegetable and fruit crops. The agent worked with each grower during the demonstration period to improve irrigation and nutrient management. Two farms were selected to host a field day to display the use of the sensors, and to inform participants about cost-share programs available through multiple sources. Forty-two people attended the field days. Exit surveys for each field day indicated that participants intend to adopt SMS (16% n=26 and 57% n=16), use irrigation scheduling (50%, n = 16) and, self-evaluate water use (50% n = 26, 57% n = 16).

Knowledge gain for these events ranged from 18% to 36%. Three of the six farmers applied for a cost-share program to acquire the sensors. For the remaining three growers, two were indifferent and one disagreed with the adoption of SMS on small, diversified farms. Three farmers adjusted their irrigation practices while using the sensors although all six farmers self-reported water savings. Additional efforts are needed to educate farmers on tools and programs available for water conservation in horticulture.

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Extending Extension's Education to Half a Million

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Research and extension face challenges communicating science to new diverse audiences, while supporting traditional clientele. Science-based education through YouTube and other social media outlets provides on-demand education that facilitates knowledge gain and behavior change. YouTube education caters to visual, auditory, and hearing-impaired learners. Transcripts are generated in over fifty languages to accommodate diverse audiences.

The demand for a popular hydroponics class exceeded the supply beginning in 2017. An easily searchable, science-based, YouTube video entitled "Hydroponic Lettuce Hannah Wooten" <https://www.youtube.com/watch?v=GQey35Tt24I> was developed in Feb. 2018 in partnership with Seminole Government Television to provide on-demand learning about hydroponics. The video supports existing clientele's adoption of practices, while expanding the reach to audiences far beyond the local extension office resulting in immense value with over 434,000 views with an average view duration of 5 out of 9 minutes. The video has increased the number of subscribers by 1533%, resulted in over 5800 "likes," and at least 350 comments—many of which commend the educational delivery of information.

As a result of the video, individuals locally, nationally, and internationally have contacted University of Florida/IFAS Extension for more science-based information about growing hydroponically. Additionally, educators who are less experienced in the subject of hydroponics rely on the YouTube video to support their own educational programs, thus extending the reach of expertise without exhausting extension agents and resources.

In conclusion, extension engagement in social media via YouTube delivers science-based information supporting existing clienteles' adoption of practices beyond the classroom, while also catering to new diverse audiences.

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Surveys for Resistance-breaking Tospoviruses in Southwest and South Florida Tomato Fields

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Viruses within the *Tospoviridae* family are highly destructive, thrips-transmitted viruses that cause significant losses of up to 100% on tomato production around the world. Therefore, using tospovirus resistant tomato varieties (with the Sw-5 gene) as part of integrated pest management is critical to reduce major losses. Sw-5 is a single dominant gene and confers resistance to tomato spotted wilt virus (TSWV), and is also effective against related tospoviruses including tomato chlorotic spot virus (TCSV) and groundnut ringspot virus (GRSV). All three of these tospovirus species have been detected in Florida tomatoes.

In 2019–20, high incidences of tospovirus-like symptoms were observed in multiple tomato fields established with Sw-5 varieties in southwest and south Florida. To assess presence of potential resistance-breaking tospoviruses, we conducted field surveys. Symptomatic samples from both Sw-5 resistant and susceptible tomato plants were collected from Collier and Miami-Dade counties. The presence of the Sw-5 resistance gene and tospovirus infection in all samples were verified by PCR and RT-PCR assays, respectively. Sequences of the NSm (non-structural movement protein) gene from 84 samples collected in 2019 were determined and known resistance-breaking amino acid motifs (C118Y and T120N) in all Florida tospovirus isolates were analyzed.

Based on sequence analysis, none of the known amino acid substations were found in any of the Florida isolates. However, a novel amino acid substitution (D17E) in the NSm protein was observed predominantly in TCSV isolates from Sw-5 resistant tomatoes. Future investigations are needed to determine if this mutation is associated with potential resistance breaking.

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