

# Suppression of the Ornamental Invasive Mexican Petunia (*Ruellia simplex*) by Native Species

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## ABSTRACT

Mexican petunia (*Ruellia simplex*) is a commonly planted herbaceous ornamental known for densely invading floodplain forests in the southeastern United States. While the use of herbicides is a typical management approach, revegetation strategies are needed not only to restore the native plant community, but also to limit reinvasion. We identified four native species appropriate for floodplain revegetation in Florida, US: bushy bluestem (*Andropogon glomeratus*), common rush (*Juncus effusus*), redbow panicgrass (*Coleataenia longifolia* sp. *longifolia*), and pinebarren goldenrod (*Solidago fistulosa*). In a controlled greenhouse container study, we investigated competition between Mexican petunia and native species compositions grown from seeds under varying hydrologic conditions and seeding densities. Results showed that number of Mexican petunia individuals and biomass decreased when seeded together with either pinebarren goldenrod or a mix of four native species (bushy bluestem, common rush, redbow panicgrass, and pinebarren goldenrod). Pinebarren goldenrod and bushy bluestem had a short establishment period (2–4 weeks), similar to Mexican petunia (2–5 weeks). Data from this container study suggests that revegetation with these native species could effectively compete with and suppress Mexican petunia in field conditions.

**Keywords:** competition, greenhouse study, invasion resistance, ornamental, revegetation, wetlands

In many scenarios, invader control is not sufficient to restore native plant communities (Kettenring and Adams 2011), but methodology for stimulating native species establishment is not well developed (Mulhouse and Galatowitsch 2003). Several studies noted that planting native species can be used for invader control (Wilson et al. 2010, Cutting et al. 2013, Khan et al. 2013), but this information is lacking for wetlands (Kettenring and Adams 2011). Active revegetation with select native species may promote native species establishment (Kettenring and Adams 2011) and diverse native species assemblages may confer the most invasion resistance (Maron and Marler 2007, Mattingly et al. 2010), but little is known about the suppressive effect of native species on invasive species during initial establishment, despite the critical nature of this stage to restoration in wetlands.

To evaluate the potential of native species to suppress invading non-native species in wetland habitats, we chose the invasive Mexican petunia (*Ruellia simplex*) and habitat-appropriate native species as our model system. Native to central Mexico, Mexican petunia was first introduced into the United States as an ornamental landscape plant

in the 1940s (Huey et al. 2007, Wunderlin and Hansen 2014). Since its introduction, Mexican petunia continues to be distributed by the ornamental plant trade in Florida (Wirth et al. 2004). Mexican petunia has spread from urban landscapes into natural areas, displacing native plant communities in floodplain forests (Langeland et al. 2008, Hupp et al. 2009). Mexican petunia is listed as a Florida Exotic Pest Plant Council Category I invasive species because it is displacing native plant communities in Florida (FLEPPC 2013); management efforts are now needed to limit invasions. Recent research has demonstrated that short-term control of Mexican petunia is straightforward (Reinhardt Adams et al. 2014), but cost-effective and efficient methods for long-term management are still unknown. While mechanical or herbicide control can reduce Mexican petunia populations, limited native species recolonization occurs, despite an intact seedbank containing diverse native species (C. Reinhardt Adams et al., University of Florida, unpub. data). Barriers to native species establishment may be a result of changes in soil characteristics including soil pH and nutrient levels (Prince 2014). Because it is unrealistic to eliminate all barriers to native species recolonization, species should be selected for active revegetation that thrive under current site conditions and suppress Mexican petunia.

When testing the “natives as weed control” approach (i.e., revegetation) with native species to control invasive species, as described by Blumenthal et al. (2003), our objective was to identify native species composition, native species seeding density, and water level conditions that favored establishment of select native species over Mexican petunia. Additionally, testing different native species compositions (single native species vs. multiple native species) could determine if establishment of a diverse native plant community will provide the most invasion resistance (Maron and Marler 2007, Mattingly et al. 2010). To that end, we observed initial competition between native species and Mexican petunia in a polyhouse for 24 weeks. We hypothesized that: 1) a high seeding density of native species would suppress Mexican petunia more than a lower seeding density; 2) Mexican petunia would be most competitive under saturated soil conditions compared to drier soil conditions; and 3) that a mix of multiple native species would suppress Mexican petunia to a greater degree than only a single native species.

## Methods

### Study Site

This study was conducted in a polyhouse at the University of Florida in Gainesville, Florida, USA under ambient temperature conditions. Temperature was recorded with a HOBO® pendant temperature data logger (UA-001-64, Onset Computer Corporation, Bourne, MA) from May 27, 2013 to November 20, 2013. Average maximum and minimum temperatures ranged from warmest in August (39.2°C and 23.9°C, respectively) to coolest in November (27.6°C and 16.7°C, respectively).

### Soil pH Determination

As soil pH may be a contributing factor in Mexican petunia invasions, we conducted a preliminary study to determine appropriate pH conditions using two water pH levels (5 and 7) and under two hydrologic levels (saturated and unsaturated), that represent different pH and elevations found in various invasions; we found that the soil pH remained constant at 7. A pH 7 was used in this study as it was the pH most commonly found at invaded field sites. We conducted soil tests at regular intervals throughout the study and found that soil pH and soil nutrient levels (P, K, Ca, Mg, Al, Fe, TKN) remained consistent.

### Species Selection

We selected locally-adapted native floodplain species commonly found in Seminole County, Florida, USA that exhibited characteristics suitable for revegetation as established by Smith et al. (A.M. Smith et al., University of Florida, unpub. data): bushy bluestem (*Andropogon glomeratus*), common rush (*Juncus effusus*), redtop panicgrass

(*Coleataenia longifolia* sp. *longifolia*), and pinebarren goldenrod (*Solidago fistulosa*). Native species seeds were collected from natural Florida populations and obtained from either Ernst Conservation Seeds (Meadville, PA) (bushy bluestem, common rush, redtop panicgrass) or The Natives, Inc. (Davenport, FL) (pinebarren goldenrod) on February 13, 2013 and stored in plastic bags in a refrigerator at 10°C until sown. Mexican petunia seeds were hand-collected from the Lake Jesup Conservation Area (Sanford, FL) in October 2012, and stored in a plastic bag in the refrigerator at 10°C. We stored seeds in plastic bags per recommendation from the seed suppliers because we wanted to mimic the same storage methods that seed suppliers typically use.

### Experimental Design

Using a replacement design, we compared hydrology (saturated and unsaturated conditions), native seeding density (low = 25 seeds/m<sup>2</sup> and high = 50 seeds/m<sup>2</sup>), and native composition (common rush + Mexican petunia, pinebarren goldenrod + Mexican petunia, and a mix of multiple native species + Mexican petunia). The native species mix consisted of equal portions of four native species: bushy bluestem, redtop panicgrass, common rush, and pinebarren goldenrod. We kept Mexican petunia seeding density constant, equivalent to the low native density treatment, to examine competition at varying native densities. The low density seeding treatment was based upon average Mexican petunia seeds found per capsule on plants in a given area in dense invasions, as well as seeding rates used by restoration practitioners.

On May 2013, we mixed 75% coarse sand and 25% Fafard #2 and filled 3.05 L injection-molded polypropylene pots (Myers Industries Lawn & Garden Group, Middlefield, OH) lined with a mesh netting to prevent mix from leaving pots. The soil mix was based upon the most current soil survey of our field site, which indicated that soil at the conservation area is comprised of 100% felda and manatee mucky fine sands (USDA NRCS 2013). Additionally, preliminary soil evaluations indicated that this soil type is characteristic of Mexican petunia-invaded soils (C. Prince et al., University of Florida, unpub. data). In order to simulate characteristic nutrient levels found in invaded soils, pots received a rate of 4.5 g/pot of Osmocote 18N-6P-12K (The Scotts Miracle-Gro Co., Marysville, OH) incorporated into the soil mix.

We randomized the pots and placed them in an 8.25 m × 1.55 m ponded bench in a plastic-sided polyhouse under ambient temperature conditions. Pots that received saturated treatments were placed directly into the ponded bench, with water levels 4 cm below the soil surface. Pots that received unsaturated treatments were placed on a single inverted 20 × 20 × 6 cm polystyrene tray (Permanest, Growers Supply Company, Dexter, MI), which kept water levels at 11 cm below the soil surface. Water levels in the ponded bench were maintained with a float and pump system (Little Giant Trough-O-Matic, Miller

Manufacturing Company, Eagan, MN). The soil in pots was allowed to saturate under the different hydrologic conditions for two days prior to addition of seeding treatments.

After accounting for seed viability to ensure anticipated number of individuals, we counted and weighed seed for native species and Mexican petunia. On May 30, 2013, seeds were sprinkled over the soil surface of designated pots. To eliminate pests from the greenhouse, we applied a pymetrozine solution (Endeavor, Syngenta, Greensboro, NC) at a rate of 74 ml/378 L of water on September 20, 2013, and an abamectin solution (Avid 0.15EC, Syngenta, Greensboro, NC) at a rate of 236 ml/378 L of water and a thiamethoxam solution (Flagship 25WG, Syngenta, Greensboro, NC) at a rate of 118 ml/378 L of water on November 7, 2013.

### Data Collection

Seedling establishment, measured as the first true leaf and total number of individuals was measured weekly for 24 weeks. Shoot height was measured biweekly for 24 weeks. Aboveground biomass and belowground biomass were collected at the conclusion of the study at week 24, by cutting the aboveground biomass at the base, sorting by species, and rinsing the belowground biomass. Biomass was oven dried at 70°C for four days then weighed.

### Statistical Analyses

The experiment consisted of a  $2 \times 2 \times 4$  randomized complete block design with hydrology and native density at two levels, and species composition at four levels. There were five replicates of each treatment combination with the exception of two treatment combinations. The two treatment combinations evaluating a high seeding density of Mexican petunia were not used as we were not interested in seeding this species at a higher rate than would be observed in the field. The model was analyzed using a normal distribution and the identity link function. We analyzed main effects in SAS (v. 9.4, SAS Institute, Cary, NC) using the PROC GLIMMIX statement to estimate means for species composition, water levels, and native density at week 24. Each species was analyzed separately for seedling establishment, number of individuals, shoot height, and biomass. We used Chi-square and DF statistics to determine model fit. Type III F-tests were used to determine F-statistics. Interactions were analyzed using a 'by level' option in the least square means statement with a 'slice' statement to evaluate pairwise comparisons. We analyzed main effects and interactions over time with hydrology and density treatments as fixed effects and week as a random statement with cs as the covariance structure. Pairwise comparisons were evaluated with a significance level of  $p = 0.05$ . Normality was checked by investigating histogram and normality plots of the conditional residuals.

## Results

### Seedling Timing and Establishment

Timing of seedling establishment was different for all species, with no differences between seeding densities and water levels for an individual species. Bushy bluestem ( $F = 6.10, p < 0.0001$ ) and pinebarren goldenrod ( $F = 6.25, p < 0.0001$ ) established first, within 2–4 weeks after sowing, but only 15% and 25% of sown seeds germinated, respectively. Common rush ( $F = 1.61, p = 0.0349$ ) and redtop panicgrass ( $F = 0.93, p = 0.5613$ ) were slower to establish; the earliest seedling establishment occurred 6–9 weeks with  $< 1\%$  germination and 3–5 weeks with  $< 1\%$  germination after sowing, respectively.

Mexican petunia had the quickest establishment period with the greatest number of germinated individuals. Seedlings established 2–5 weeks after sowing in all treatments, with 62% germination. Mexican petunia had the most rapid seedling establishment regardless of native species composition ( $F = 6.14, p = 0.0011$ ) when it was in saturated conditions ( $F = 166.23, p < 0.0001$ ).

### Total Number of Individuals

Even though viable seeds were sown to achieve either eight (25 seeds/m<sup>2</sup>) or 16 (50 seeds/m<sup>2</sup>) individuals in the low and high density treatments respectively, under no conditions did all seeded individuals germinate. For bushy bluestem ( $F = 0.42, p = 0.5263$ ), common rush ( $F = 0.84, p = 0.3665$ ), and redtop panicgrass ( $F = 0.31, p = 0.5841$ ), the number of germinated individuals did not differ with seeding densities, water levels, or species compositions. For most native species, no more than three individuals germinated per pot, with the exception of redtop panicgrass that had one individual per pot or no germination.

Conversely, number of individuals for Mexican petunia varied amongst treatments (Table 1). Mexican petunia germinated at higher rates when seeded alone under saturated conditions or with common rush; both scenarios resulted in 75% germination of Mexican petunia. While highest germination was found in these treatments, we also found that Mexican petunia had significantly lowest germination when seeded with pinebarren goldenrod (25% germination) or a native species mix (23% germination).

### Impact on Mexican Petunia Plant Height and Biomass

Shoot height of Mexican petunia was affected only by water levels (Figure 1). Mexican petunia was tallest under saturated conditions and shortest in unsaturated conditions ( $F = 95.07, p < 0.0001$ ). Mexican petunia shoot height was not impacted by native species composition or density ( $F = 0.06, p = 0.9392$ ).

Biomass of Mexican petunia varied significantly between water levels for shoot ( $F = 103.31, p < 0.0001$ ) and root

**Table 1.** Test statistics from the model for total number of Mexican petunia individuals for each native species composition treatments, native species densities, and water levels at 24 weeks. The mean, standard error (SE), degrees of freedom (df), t-statistic (t), and p-value for each interaction is shown. Treatments are separated by a horizontal line. Means within each treatment grouping followed by the same letter were not significantly different ( $p = 0.05$ ).

Treatment		Mean	SE	df	t	p-value
<i>Mexican petunia alone</i>						
	saturated	6.11 <sup>a</sup>	0.51	56	13.11	< 0.0001
	unsaturated	0.29 <sup>b</sup>	0.24	56	0.63	0.5338
<i>common rush + Mexican petunia</i>						
	saturated—low	5.63 <sup>a</sup>	0.80	56	8.03	< 0.0001
	saturated—high	6.55 <sup>a</sup>	1.01	56	9.44	< 0.0001
	unsaturated—low	0.60 <sup>b</sup>	0.58	56	0.42	0.9753
	unsaturated—high	0.33 <sup>b</sup>	0.24	56	1.40	0.5022
<i>pinebarren goldenrod + Mexican petunia</i>						
	saturated—low	2.38 <sup>b</sup>	0.60	56	2.66	0.0490
	saturated—high	4.58 <sup>a</sup>	0.37	56	3.34	0.0079
	unsaturated—low	0.63 <sup>c</sup>	0.40	56	6.00	< 0.0001
	unsaturated—high	0.55 <sup>c</sup>	0.37	56	6.11	< 0.0001
<i>native species mix + Mexican petunia</i>						
	saturated—low	4.92 <sup>a</sup>	0.51	56	7.48	< 0.0001
	saturated—high	2.15 <sup>b</sup>	0.87	56	3.26	0.0099
	unsaturated—low	0.48 <sup>bc</sup>	0.24	56	0.73	0.8831
	unsaturated—high	0.00 <sup>c</sup>	0.00	56	0.00	1.0000
Native species composition						
<i>Mexican petunia alone</i>		3.20 <sup>a</sup>	0.33	56	9.71	< 0.0001
<i>common rush + Mexican petunia</i>		3.28 <sup>a</sup>	0.23	56	14.08	< 0.0001
<i>pinebarren goldenrod + Mexican petunia</i>		2.03 <sup>b</sup>	0.23	56	8.72	< 0.0001
<i>native species mix + Mexican petunia</i>		1.89 <sup>b</sup>	0.23	56	8.11	< 0.0001

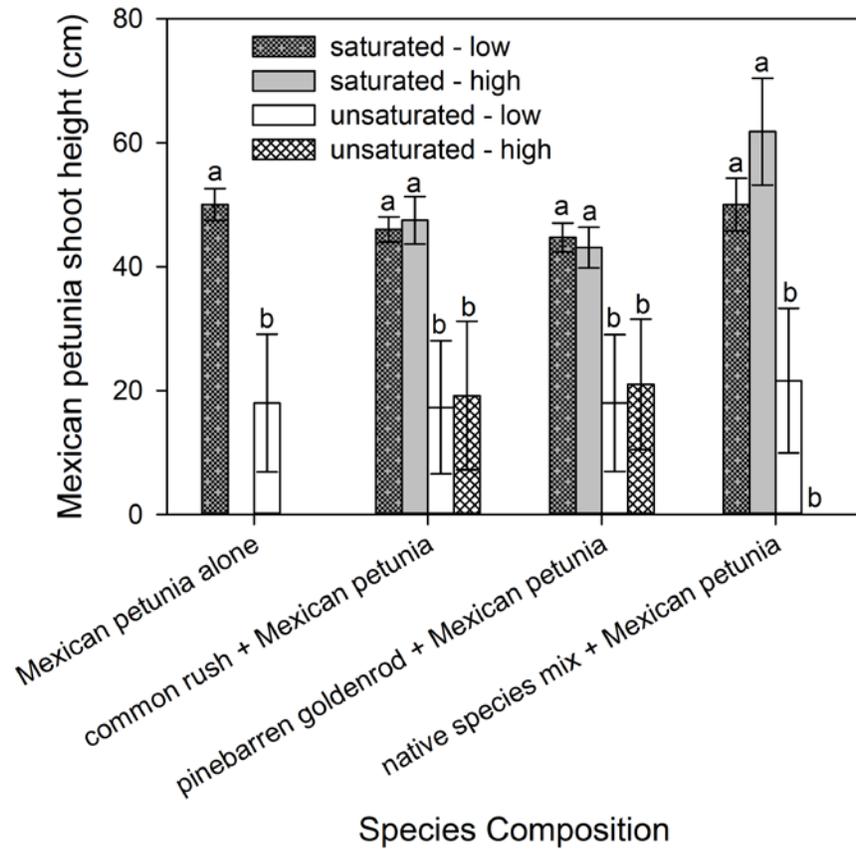
biomass ( $F = 21.93$ ,  $p < 0.0001$ ); it also varied between native species composition treatments for total biomass ( $F = 10.86$ ,  $p < 0.0001$ ; Figure 2). Mexican petunia biomass was similar between native species density treatments. Shoot and root biomass of Mexican petunia was higher under saturated conditions. Mexican petunia produced less total biomass when grown with pinebarren goldenrod or a native species mix ( $F = 10.86$ ,  $p < 0.0001$ ; Figure 2C), than when grown alone or with common rush. Mexican petunia did not germinate in the unsaturated treatment when planted with a native species mix.

## Discussion and Management Implications

With many invasive species, particularly in degraded landscapes, effective short-term control methods can be overcome by a lack of native species establishment and subsequent reinvasion (Kettenring and Adams 2011, Alday et al. 2013, Reinhardt Adams et al. 2014). Active revegetation is one approach to potentially achieve long-term control. Results from greenhouse studies can inform appropriate choices regarding native species selection, seeding densities, and anticipated competitive relationships under field scenarios increasing the likelihood of success under field conditions. This study demonstrated that certain native species may hasten restoration of the native plant

community through quick germination and establishment rates, while limiting reinvasion under simulated field conditions. Specifically, in formerly-invaded floodplains, pinebarren goldenrod and a mix of native species may be able to out-compete Mexican petunia seedlings by decreasing Mexican petunia germination (Table 1) and growth (Figure 2).

Rapid initial establishment of Mexican petunia likely contributes to successful invasion, making rapid establishment of native species following control crucial to native species survival. Many invasive plants exhibit rapid germination rates (Wainwright and Cleland 2013). Mexican petunia is known for rapid germination under a variety of environmental conditions (Wilson et al. 2004), often with faster establishment rates than accompanying native species. In our study, rapid establishment of Mexican petunia seedlings within 2–5 weeks after sowing suggests that high germination and fast establishment rates will likely out-compete many slower-to-germinate native species. In this study, pinebarren goldenrod and bushy bluestem (as part of the native species mix) established during this 2–5 week period. We saw reductions in number of Mexican petunia individuals when sown with these two fast establishing native species. While common rush and redtop panicgrass also germinated during this critical time frame, < 1% of seeds germinated, and no reduction in Mexican petunia individuals was observed when planted with these



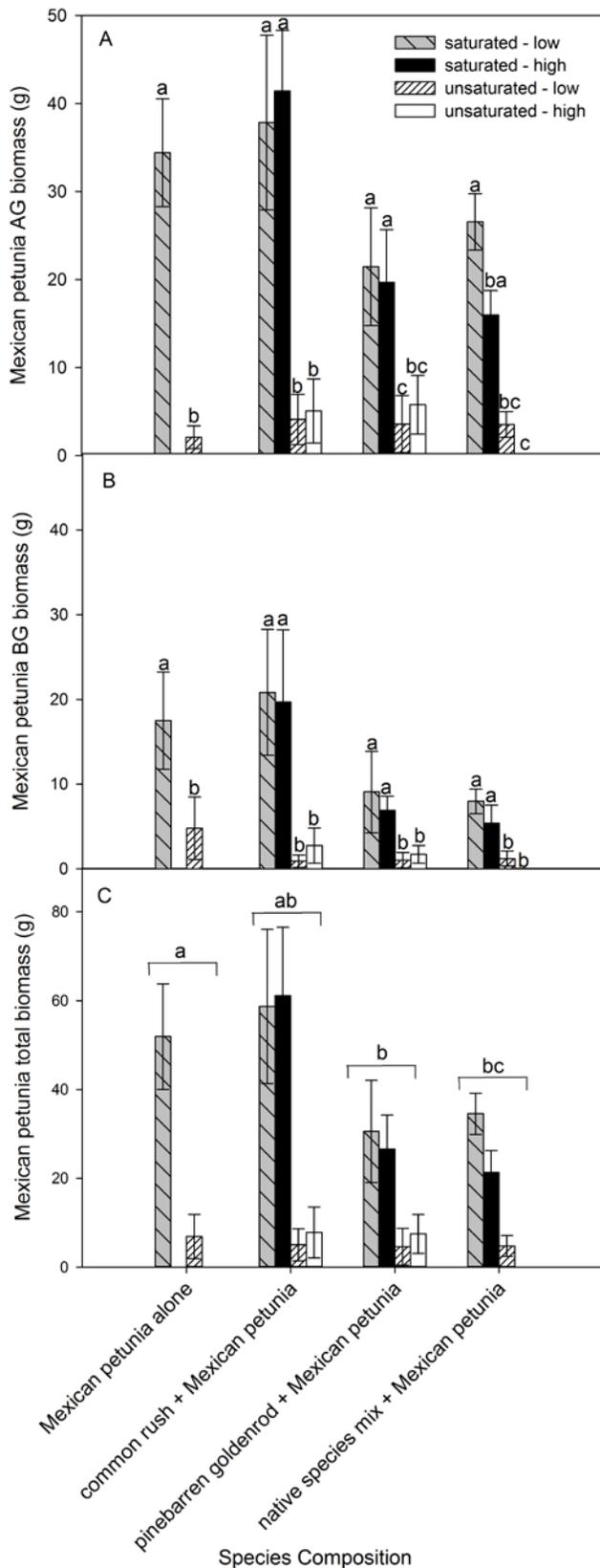
**Figure 1.** Final mean shoot height (cm) of Mexican petunia in each treatment at the end of 24 weeks. Means with the same letter were not significantly different ( $p = 0.05$ ).

species. As a result of the low germination of common rush, Mexican petunia readily germinated (75% germination) and established; however, we expected that Mexican petunia would have high germination when planted with common rush since common rush had low total germination. Surprisingly, even though Mexican petunia has rapid germination and establishment rates, we saw no germination of Mexican petunia when planted with the native species mix in the unsaturated treatment (Table 1), suggesting that seeding native species with fast establishment rates in drier soil conditions may limit Mexican petunia germination and subsequent establishment. Ruwanza and colleagues (2013) similarly found that native species with fast establishment rates were able to successfully establish and persist in natural areas when planted as part of revegetation efforts. Our results confirm what previous researchers have found; a rapid initial establishment rate for native species in areas with plant invasions is critical for native species to compete with invasive species (Jessop and Anderson 2007, Hata et al. 2012).

While rapid establishment is a key selection criteria for native species chosen for revegetation, our results also suggest that pinebarren goldenrod and a native species mix will better compete with Mexican petunia. More specifically, planting the native species mix may allow for some native

species to readily germinate and persist prior to reinvasion (r selected species), while other native species are slower to germinate but may be more successful in establishing in these newly revegetated areas (k selected species) (Parkinson et al. 2013). Pioneer species (r) are known to be fast growing and short-lived, whereas later successional species (k) are more slow growing but more resilient in frequently flooded floodplains (Bashforth et al. 2011). While all of our native species are later successional species, it is possible that those with faster germination rates may germinate and persist, and compete against Mexican petunia, before the remaining native species germinate.

We measured competition at the outset of establishment, measured as emergence, but we note that invasive species suppression may also be observed as a reduction in biomass of the invader. In other plant invasions, reduction in invasive species biomass as a result of competition with native species can limit spread by keeping microsites open for native species natural recolonization (Ammond and Litton 2012, Kawaletz et al. 2013, Khan et al. 2013). In this study, we showed that Mexican petunia biomass also decreased during a 24 week period when planted with rapidly establishing native species. Functional or native species diversity, in general, may also have influenced biomass suppression. When planted with pinebarren goldenrod or the native



**Figure 2.** Mean Mexican petunia (A) aboveground [AG], (B) belowground [BG], and (C) total biomass (g) in each treatment at the end of 24 weeks. Means in the same graph with the same letter were not significantly different ( $p = 0.05$ ).

species mix, total biomass of Mexican petunia decreased, compared to Mexican petunia seeded alone. Ammond and Litton (2012) demonstrated that high functional diversity of native species, (e.g., canopy tree, shrub, groundcover species) suppressed guineagrass (*Urochloa maxima*) biomass, and ultimately limited guineagrass invasions more effectively than single species plantings. More broadly, it has been examined that revegetating with multiple native species may limit invasibility: some studies noted that planting a diverse suite of native species provided strong invasion resistance, which then promoted native plant establishment (Maron and Marler 2007, Mattingly et al. 2010). Similarly, our study found that the native species mix and pinebarren goldenrod, with functional characteristics different from Mexican petunia, suppressed Mexican petunia total biomass (Figure 2C), suggesting that these revegetation treatments may be enough to limit invasions and that diverse native plantings may provide resistance to reinvasion. Because varying levels of functional diversity existed, reduced total Mexican petunia biomass was likely a result of competition for aboveground (i.e., light, space) and belowground resources. Previous studies have noted that light availability impacts invasive species abundance (Perry and Galatowitsch 2006, Flory et al. 2007, Spellman and Wurtz 2011, Chen et al. 2013, Skalova et al. 2013), so functional diversity may have reduced light availability and limited Mexican petunia germination.

The high density native seeding was selected to be high enough to suppress Mexican petunia, but low enough to be cost effective and logistically feasible. Differences in seeding densities did not generally suppress Mexican petunia as anticipated. We saw no differences in number of individuals for native species in all treatments; however, it is possible that Mexican petunia germination and biomass would have been further reduced if higher densities of native individuals were present. Low viability of these seed lots confirmed by Smith et al. (A.M. Smith et al., University of Florida, unpub. data) were used to develop the seeding rate, and therefore could not have influenced native species germination. However, we do note that the use of recommended seed storage methods, e.g., storage in plastic bags, may have contributed to poor germination. Additional research may reveal that a higher threshold density of native species seeds may suppresses Mexican petunia. If this threshold density is cost-feasible it may ultimately better provide management recommendations.

Our work confirms a strong hydrologic preference of Mexican petunia. Mexican petunia had both higher germination and more rapid seedling establishment under saturated soil conditions. Field studies show Mexican petunia consistently occurs in an elevational gradient between submersed soil conditions and dry upland conditions, with rapid germination of seeds in saturated soil conditions in frequently inundated floodplains (Hupp 2007). These observations suggest that management of Mexican

petunia may be confined to saturated soil conditions, as drier conditions will facilitate establishment of other species. However, since Mexican petunia individuals are also occasionally found in dryer soil conditions, multiple factors, rather than hydrology alone, may better explain the spatial distribution of these invasions.

Initial competition between select native species and an ornamental invasive species in controlled conditions can inform initial invader suppression and guide revegetation efforts. In addition, consistent use of Mexican petunia in homeowners' landscapes is likely leading to frequent dispersal of propagules through stormwater runoff in Florida, thereby heightening the importance for development of revegetation plans that can control Mexican petunia and limit reinvasion. By testing revegetation strategies on a smaller scale in the greenhouse, we have shown that suppression of Mexican petunia can be achieved by seeding pinebarren goldenrod or a mix of native species in saturated soils in controlled conditions. Integration of scientific research with practical recommendations is required to address native species introductions (Holzel et al. 2012), so testing logistically and economically feasible approaches that aid practitioners is needed. Experimentally testing revegetation approaches in controlled greenhouse conditions can aid in the development of practical guidelines, but research is needed to determine the success of these guidelines under field conditions.

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