Germination of coastalplain honeycombhead (*Balduina angustifolia*) in response to photoperiod, temperature and gibberellic acid

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Abstract

Coastalplain honeycombhead (*Balduina angustifolia*) is underutilised in ecosystem restoration and pollinator conservation projects. Seeds collected from different populations throughout Florida, USA during 2007 and 2016-2018 were used in experiments that evaluated the effects of a 0 or 12-hour photoperiod, seasonal and standard temperatures, 0-5,000 ppm gibberellic acid (GA) or source population (northwest, central and south Florida) on seed germination. For central Florida seeds, germination was high (62-74%) in all seasonal temperatures except for summer (4%) and was not affected by photoperiod. Germination differed for two northwest populations and was higher in warm (35/25 and 30/20°C) temperatures compared with cool (25/15 and 20/10°C) temperatures (63-72% vs. 9-36%). In a one-year move-along experiment, germination was similar at all seasonal and constant temperatures for seeds from central, south-central and south Florida, while northwest Florida seeds had reduced germination under winter temperatures. Germination of northwest Florida seeds after exposure to GA increased for two of four and three of four populations for one year and five-day old seeds, respectively. There were different temperature requirements on a regional scale and different dormancy levels at a local scale for coastalplain honeycombhead.

Keywords: coastal dune, coastal plain hesperapis, gulf coast solitary bee, *Hesperapis oraria*, physiological dormancy

Introduction

Coastalplain honeycombhead [*Balduina angustifolia* (Pursh) B. L. Robinson (Asteraceae)], hereafter referred to as honeycombhead, is a forb native to the Southeastern USA (USDA, 2020). It is found in sunny, dry and nutrient poor areas such as beach dunes, coastal grasslands, scrubby flatwoods, scrub and disturbed areas (Parker and Jones, 1975; FNAI,

2010; Wunderlin and Hansen, 2011). Honeycombhead has yellow ray and disk flowers at anthesis during autumn which provide crucial resources for a vast array of pollinators including butterflies, bees, flies, wasps and beetles, and their seeds provide resources for birds and ants (Parker and Jones, 1975; Deyrup and Menges, 1997; Glassberg *et al*., 2000; Stephens, 2013; Stephens and Quintana-Ascencio, 2015). At a single site in south Florida (Archbold Biological Station, Venus, Florida), 39 species of bees were documented visiting their flowers (Deyrup *et al*., 2000). Moreover, the endemic, threatened, solitary and ground-dwelling bee, coastal plain hesperapis (*Hesperapis oraria* Snelling and Stage), is dependent on honeycombhead flowers for survival, only emerging from the ground a few weeks each year in September to October to collect pollen and nectar (Cane *et al*., 1996; Hunsburger, 2013; Davis *et al*., 2020). The known range of the coastal plain hesperapis is restricted to areas where honeycombhead patches of specific character and size are found on coastal mainland and barrier islands of the Florida panhandle (Davis *et al*., 2020).

Coastalplain honeycombhead is widely distributed throughout Florida and a few counties in coastal Alabama, coastal Mississippi and southern Georgia (USDA, 2020; Wunderlin *et al*., 2020). This range includes climates with frequent freezes in the north portion of its range (Zone 8a where it is reportedly an annual) and climates with rare to no freezes in the south portion of its range (Zone 11a where it is reportedly a biennial) (figure 1; Parker and Jones, 1975; Anderson and Menges, 1997; USDA, 2012). It is possible that life cycle characteristics for coastalplain honeycombhead differ across its range as has been recorded for other species in the Asteraceae (Encinas-Viso *et al.*, 2020). This difference could be in response to cold temperatures, as plants die in response to freezes (Thetford, pers. obs.) and may be influenced by seed germination requirements (e.g., optimal germination temperatures) and/or seed dormancy mechanisms which inhibit germination until the appropriate season. Understanding the germination strategy of coastalplain honeycombhead could provide insight into the mechanisms of different life history traits across its range and could have important practical implications for applied conservation and restoration strategies.

Germination requirements and dormancy characteristics of honeycombhead are not well-known. Achenes of honeycombhead, referred to as seeds, collected from central Florida (Archbold Biological Station) were exposed to three different burial depths (0, 5 and 20 mm of sand) within pots and higher emergence occurred when buried with 0 and 5 mm sand than with 20 mm sand (~15% vs. ~1%) (Petrů and Menges, 2004). Seeds collected in south central Florida (Lake Wales Ridge) during autumn (September to October) and placed in a field experiment within weeks after collection had reduced emergence proportion as seed density increased, lower emergence in intact vs. a degraded habitat, and increased emergence in bare sand compared with under shrubs or in leaf litter (Stephens and Quintana-Ascencio, 2015). Germination of the same seeds a few weeks after seed collection within growth chambers set at seasonal temperatures and photoperiods resulted in 38% germination (Stephens, 2013). Studies to date have yielded low germination or emergence (≤ 38%; Petrů and Menges, 2004; Stephens, 2013; Stephens and Quintana-Ascencio, 2015) and it is unclear if this is due to low seed viability in test populations or an unrecognised dormancy within a proportion of seeds being tested.

Moreover, germination requirements and dormancy characteristics of other populations of honeycombhead, particularly of Gulf coastal populations important for the threatened bee, coastal plain hesperapis, remain largely unknown.

Coastalplain honeycombhead is an important candidate for use in ecosystem restoration projects, particularly in areas of high pollinator conservation value. Use of this plant in restoration projects is limited and little is known about its basic seed germination biology. Here we describe the seed viability, germination requirements and seed dormancy characteristics for coastalplain honeycombhead across its range in Florida. We tested the effects of photoperiod, temperature, gibberellic acid and population (collection location) on the germination of honeycombhead from across Florida.

Materials and methods

A series of germination experiments were conducted on coastalplain honeycombhead seeds from populations across Florida and during different years (table 1, figure 1). Dry, brown to black capitula were collected from wild plants, air-dried for at least two weeks, and placed in air-tight containers in the dark at room temperature (approximately 24°C) in an air-conditioned laboratory until experiment initiation. Each seed source was preconditioned by visually grading fruits (single-seeded achenes, hereafter referred to seeds) and only normal seeds (plump conical shaped seeds with no sign of herbivory) (figure 2A) were used for experimentation, while abnormal seeds [seeds with herbivory and t-shaped (deformed) or sunken (empty) seeds] were discarded (figure 2B-D). Seed source regions are identified in experiment headings for clarity hereafter.
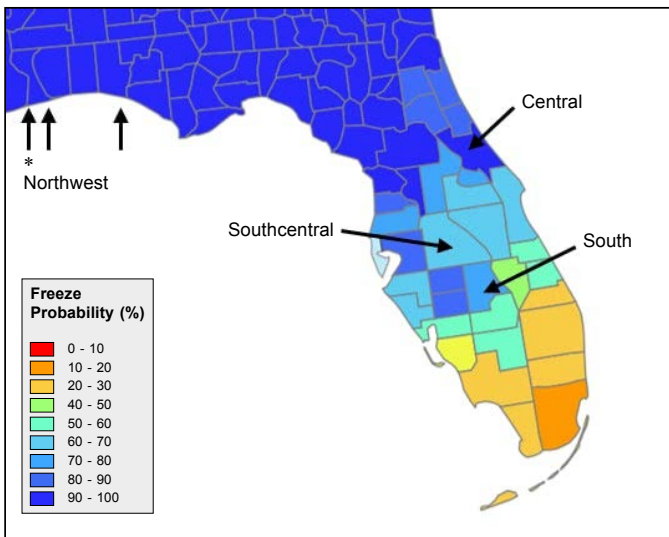

Figure 1. Freeze probability map for regions where *Balduina angustifolia* (coastalplain honeycombhead) seeds were collected. The asterisk (*) denotes an area with two collection locations that were too close to each other to warrant individual arrows. Map was obtained from agroclimate.org.

Table 1. Seed population, region within the state of Florida (USA), coordinates and date of seed collection for populations of coastalplain honeycombhead (*Balduina angustifolia*) seeds used in various experiments.

Population	Region	Coordinates (North, West)	Collection date	Experiments[1]
Archbold	South	27.182804, 81.352935	Late-Nov. 2018	2018 move-along
Bok Tower	Southcentral	27.936872, 81.577313	Late-Nov. 2018	2018 move-along
Scrub Oak Preserve	Central	28.986070, 81.333848	Early-Jan. 2007	Seasonal temperature and photoperiod; 2007 move-along
Deer Lake State Park	Northwest	30.301423, 86.078656	12 Dec. 2018	Fresh seeds GA; stored seeds GA
Navarre Beach	Northwest	30.378181, 86.897092	12 Dec. 2018	2018 move-along; fresh seeds GA; stored seeds GA
Navarre Beach	Northwest	30.378181, 86.897092	Fall (Nov. to Dec.) 2016	Standard temperature and population
Big Lagoon State Park	Northwest	30.311511, 87.418825	12 Dec. 2018	Fresh seeds GA; stored seeds GA
Perdido Key	Northwest	30.295612, 87.447959	12 Dec. 2018	Fresh seeds GA; stored seeds GA
Perdido Key	Northwest	30.295612, 87.447959	Fall (Nov. to Dec.) 2016	Standard temperature and population

[1] Experiments were initiated on the following dates: 19 January 2007 (2007 move-along), 24 January 2017 (seasonal temperature and photoperiod), 18 September 2017 (standard temperature), 11 January 2018 (2018 move-along), 17 December 2018 (fresh seeds GA), 8 September 2019 (photoperiod), 17 December 2019 (stored seeds GA).
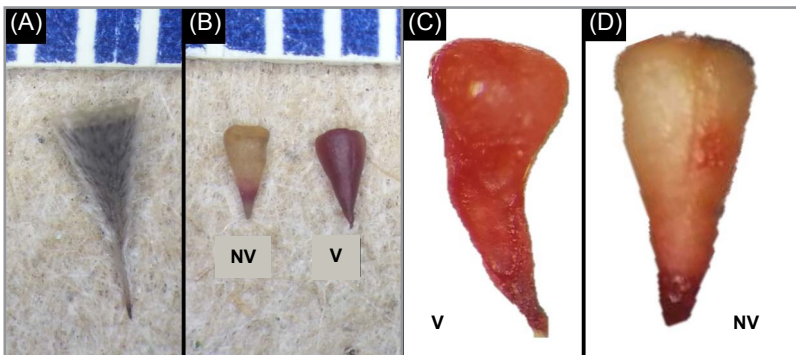

Figure 2. Achene (seed) (**A**) and examples of positive and negative tetrazolium viability staining (**B-D**) of *Balduina angustifolia* (coastalplain honeycombhead) seeds with outer layer removed. Seeds were exposed to a 1% TZ solution overnight at 37°C. Note the 1 mm wide bars for reference in (A) and (B). V = viable, while NV = non-viable staining patterns.

Seeds were considered germinated when the radicle protruded through the pericarp > 1 mm. Experiments used randomised complete block designs with full factorial arrangements of treatments where position in the chamber (shelf number) was treated as a blocking factor. Seeds were surface-sterilised immediately before the start of each experiment by submergence in a 1.2% sodium hypochlorite solution for 10 minutes and then triple-rinsed with deionised and distilled water. Seeds were placed within transparent germination boxes (110 × 110 × 140 mm, Hoffman Manufacturing, Corvallis, OR), on one sheet of germination paper atop of one sheet of blotter paper (Hoffman Manufacturing) saturated with 15 mL of distilled deionised water. Germination boxes were placed in a growth chamber (Percival Scientific, Model I30VL, Perry, IA). Except for seeds in the dark treatment in the seasonal temperature and photoperiod experiment which were observed once at the end of the experiment, seeds were observed 3-times weekly and germinated seeds were removed and recorded.

Seasonal temperatures and photoperiod (central Florida)
On 24 January 2007, an experiment (four × two full factorial arrangement of treatments) was initiated testing the effects of seasonal temperatures [winter (22/11°C), early-spring or late-autumn (27/15°C), early-autumn or late-spring (29/19°C) and summer (33/24°C)] and photoperiod (12 or 0 hours of light per day), and their interactions on germination. Seeds in this experiment were collected from plants within Scrub Oak Preserve in central Florida during January of 2007 (table 1). Temperatures represented the seasonal averages of Florida's seasons for the respective seasons (Pérez and Kettner, 2013). Daily high temperatures coincided with light exposure. Four germination boxes, each with 25 seeds, were used per temperature × photoperiod treatment combination. Germination data was collected 28 days after sowing.

Standard temperature and population (Northwest Florida)
On 18 September 2017, an experiment (four × two full factorial arrangement of treatments) was initiated testing the effects of temperature (20/10, 25/15, 30/20 and 35/25°C day/night), population (Navarre Beach and Perdido Key) and their interactions on the germination of honeycombhead seeds collected from plants in coastal Northwest Florida during autumn of 2016 (table 1). Temperatures were chosen because of their widespread use in standard germination tests (Baskin and Baskin, 2014). Seeds were placed into growth chambers with a 12-hour photoperiod (57 ± 7 µmol m^{-2} second^{-1} cool-white fluorescent light) with daily high temperatures coinciding with light exposure. Four germination boxes, each with 50 seeds, were used per temperature × population treatment. Seeds were observed 3-times weekly, and water was added as needed to maintain blotter and germination paper saturation. Germination data was collected 28 days after sowing.

Move-along experiments
Move-along experiments help to determine whether cold stratification, warm stratification or a sequence of both is required to break dormancy (Baskin and Baskin, 2003; table 2). Two move-along experiments, in 2007 and 2018, were conducted using seeds collected from different sites and in different years (table 1). Temperature treatments included four

Table 2. Temperature shifts across time used in the move-along experiments and the seasons they were intended to mimic. Adapted from Baskin and Baskin (2003). Temperatures used represent the seasonal averages of Florida's seasons and were determined by averaging air temperatures from throughout the state (Pérez and Kettner, 2013).

Number of weeks	Weeks after sowing	Temperature (°C)*	Season
Constant seasonal temperatures			
56	–	22 / 11	Winter
56	–	27 / 15	Early-spring / late-autumn
56	–	29 / 19	Early-autumn / late-spring
56	–	33 / 24	Summer
Move-along summer treatment (MAS)			
12	12	33 / 24	Summer
4	16	29 / 19	Early-autumn
4	20	27 / 15	Late-autumn
12	32	22 / 11	Winter
4	36	27 / 15	Early-spring
4	40	29 / 19	Late-spring
12	52	33 / 24	Summer
Move-along winter treatment (MAW)			
12	12	22 / 11	Winter
4	16	27 / 15	Early-spring
4	20	29 / 19	Late-spring
12	32	33 / 24	Summer
4	36	29 / 19	Early-autumn
4	40	27 / 15	Late-autumn
12	52	22 / 11	Winter

* 12 hours at high temperature / 12 hours at low temperature.

seasonal constant temperatures [winter (22/11°C), early-spring or late-autumn (27/15°C), early-autumn or late-spring (29/19°C) and summer (33/24°C)] and two move-along temperatures that progress over time mimicking seasonal temperature changes starting in summer (MAS) or winter (MAW). Temperature treatments are shown in table 2 and coincided with Florida seasonal temperature averages (Perez and Kettner, 2013). Seeds were placed in growth chambers with a 12-hour photoperiod with daily high temperatures coinciding with light exposure. Four germination boxes (2007) or Petri dishes (2018), each with 25 seeds, were used per treatment (n = 24 boxes per population) and germination was recorded weekly for 52 weeks.

The 2007 experiment was initiated 1 January 2007 and tested the effects of temperatures (four constants and two move-along temperatures) on germination of seeds collected in early 2007 from central Florida (table 1). Seeds were incubated in 60 mm-diameter × 15 mm-deep glass Petri dishes. The 2018 experiment was initiated on 11 January 2018 and tested the effects of temperatures (four constants and two move-along temperatures), regional populations [northwest Florida (Navarre Beach), southcentral Florida (Bok Tower, Lake Wales, Florida) and south Florida (Archbold)], and their interactions

on seed germination. The experiment had a full factorial arrangement of treatments. The experiment used seeds collected in late 2017 (table 1). Seeds were incubated in germination boxes and a 2 mL L^{-1} plant preservative mixture (PPM) solution (Plant Cell Technology, Washington, D.C.) was added to autoclaved, distilled water after week-4 to manage high rates of disease observed in germination boxes. A subsample of seeds (100 seeds) from each population was subjected to a tetrazolium (TZ) test to determine initial viability. Viability tests were conducted by exposing seeds to a 1% TZ solution overnight at 37°C and seeds were considered viable if stained evenly red and non-viable if staining patterns were otherwise (figure 2B-D).

Gibberellic acid experiments (*northwest Florida*)
Two experiments were conducted on fresh and 1-year old seeds from four populations to determine the effects of GA, population (Deer Lake State Park, Navarre Beach, Big Lagoon State Park and Perdido Key; table 1) and their interactions on seed germination. Seeds were soaked overnight (approximately 16 hours) in GA [GA_3 (> 93.0% purity), Plantmedia, Dublin, Ohio] solutions of 0-500 ppm. Seeds were surface-sterilised prior to GA application, placed in a growth chamber set to 29/19°C day/night temperatures and exposed to a 12-hour photoperiod with daily high temperatures coinciding with light exposure. The experiments had a full factorial arrangement of treatments. Germinated seeds were counted 3-times weekly, diseased seeds removed, and final germination was recorded 28 days after sowing. Germination was calculated as (number of seeds germinated / (total number of seeds – diseased seeds)).

On 17 December 2018, fresh seeds (five days after seed collection; table 1) of each population were subjected to GA treatments of 0, 125, 250 or 500 ppm. Three germination boxes, each with 50 seeds, were used per concentration × population treatment combination (n = 48 boxes). A subsample of seeds (100 seeds) from each population was subjected to a tetrazolium (TZ) viability test as described above.

On 17 December 2019, stored seeds of each population were subjected to GA treatments of 0 or 500 ppm. Seeds were stored in air-tight jars at ambient laboratory conditions (~24°C and RH ~30%) in the dark for 370 days after seed collection (table 1). Four replicate germination boxes, each with 50 seeds, were used per treatment (n = 32 boxes).

Statistical analysis
Main effects and their interactions on the final germination proportions for all experiments were tested by fitting linear mixed models (PROC glimmix with identity link function in SAS 9.4.). Germination boxes or Petri dishes were designated as random effects. A Kenward-Rogers approximation was used for computing the denominator degrees of freedom for the fixed effects tests. All analyses were performed using SAS 9.4. Means comparisons were obtained using the lsmeans statement. Move-along experiments included a repeated measures statement and the slice statement was used to make simple comparisons of GA effects within locations for both GA studies. The germination percentage of seeds per germination box or Petri dish was calculated and a mean and standard error of the mean were calculated within each experiment.

Results

Temperature and photoperiod experiments
The seasonal temperature and photoperiod experiment showed that temperature influenced the germination of seeds from central Florida, and that the response was independent of photoperiod (table 3). Germination was high (62-74%) for early-autumn or late-spring (29/19°C), early-spring or late-autumn (27/15°C), and winter (22/11°C) seasonal temperatures compared with the summer temperature (33/24°C) which had only 4% germination (figure 3). Mean germination was similar across temperatures in light (55%) and dark (48%).

Table 3. Type III sums of squares for the main effects and their interactions on the germination of seeds of *Balduina angustifolia* (coastalplain honeycombhead) in the photoperiod, seasonal temperature and photoperiod standard temperature, 2007 move-along, 2018 move-along, fresh seeds GA and stored seeds GA experiments.

Effect	Numerator DF	Denominator DF	F-value	*P*-value
Seasonal temperature and photoperiod experiment				
Temperature	3	24	65.16	<0.0001
Photoperiod	1	24	2.88	0.1025
Temperature × Photoperiod	3	24	0.37	0.7733
Standard temperature and population experiment				
Temperature	3	21	9.26	<0.0001
Population	1	21	2.32	0.0108
Temperature × Population	3	21	4.77	0.4137
2007 move-along experiment				
Week	1	32.32	137.68	<0.0001
Temperature	5	32.32	32.05	<0.0001
Week × Temperature	5	32.32	17.53	<0.0001
2018 move-along experiment				
Week	1	53.97	271.68	<0.0001
Temperature	5	51.91	17.81	<0.0001
Week × Temperature	5	53.97	11.36	<0.0001
Population	2	51.91	17.00	<0.0001
Week × Population	2	53.97	1.52	0.2276
Population × Temperature	10	51.91	4.72	<0.0001
Week × Population × Temperature	10	53.97	2.21	0.0311
Fresh seeds GA experiment				
Gibberellic acid	3	30	308.52	<0.0001
Population	3	30	49.51	<0.0001
Gibberellic acid × Population	9	30	12.60	<0.0001
Stored seeds GA experiment				
Gibberellic acid	1	21	95.80	<0.0001
Population	3	21	20.43	<0.0001
Gibberellic acid × Population	3	21	11.14	<0.0001

Temperature and population both influenced germination of seeds collected from Navarre Beach and Perdido Key in northwest Florida in the Standard temperature and population experiment (table 3). There was not a significant interaction ($P = 0.4137$) of these main effects, indicating the responses were independent. The highest germination (63-72%) was observed for seeds exposed to the two warm season temperatures (30/20 and 35/25°C) compared with cooler temperatures (figure 4). Higher germination was observed for 25/15°C than for 20/10°C (36 vs. 8%). Across temperatures, germination was higher for seeds collected from Navarre Beach (50%) compared with Perdido Key (39%).
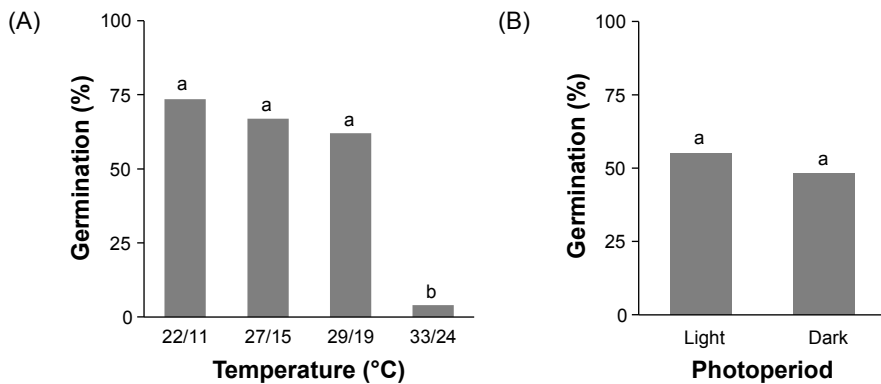
Figure 3. Mean germination of *Balduina angustifolia* (coastalplain honeycombhead) seeds collected from central Florida in January 2007. (**A**) Temperature regimes represent winter (22/11°C), early-spring or late-autumn (27/15°C), early-autumn or late-spring (29/19°C) and summer (33/24°C). (**B**) Seeds were placed in growth chambers with day/night temperatures with a 0 or 12-hour day photoperiod corresponding to daily high temperatures. Different letters above columns within each graph represent differences in means determined using LSmeans (at α = 0.05).

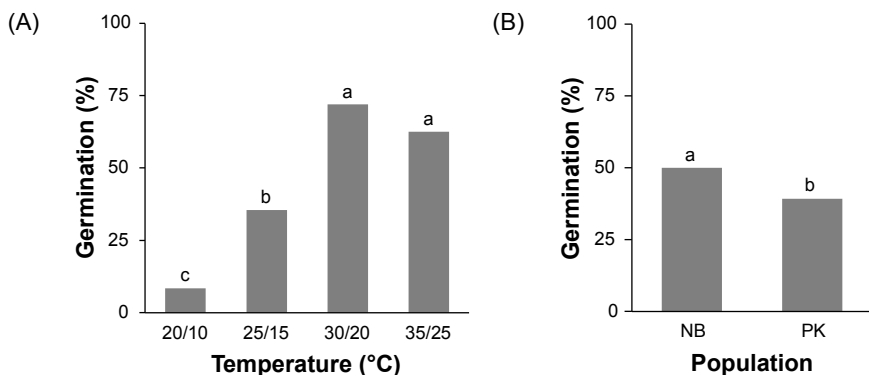Figure 4. Effects of temperature (**A**) and population (**B**) on germination of *Balduina angustifolia* (coastalplain honeycombhead) collected from coastal northwest Florida in the fall of 2018 (population NB = Navarre Beach and PK = Perdido Key). Seeds were placed in growth chambers with day/night temperatures with a 12-hour day photoperiod (57 ± 7 µmol m^{-2} second^{-1} cool-white fluorescent light) corresponding to high temperatures. Different letters above bars within each graph represent differences in means determined using LSmeans (at α = 0.05).

Move-along experiments

In the 2007 move-along experiment with seeds from central Florida, the main effects of time after sowing (week) and temperature both contributed significantly to the germination response and a significant interaction of week × temperature indicated the germination response over time was not the same at all temperatures (table 3). At week-4, germination was higher (59-80%) for cooler temperatures (22/11, 27/15, 29/19°C) and MAW compared with warmer temperatures (33/2°C) and MAS (figure 5). Germination of MAS increased similarly to the 33/24°C control until exposed to 27/15°C when germination increased and then reached the maximum once exposed to 22/11°C. By week 52, germination was similar (72-90%) among all temperatures tested and the maximum germination for cooler temperatures at 52 weeks had already been achieved at week-4.

In the 2018 move-along experiment, pre-germination viability was low for the south Florida population (50%) and high for the southcentral (94%) and northwest (88%) Florida populations. The main effects of time after sowing (week), temperature, and population all contributed significantly to the germination response and a significant interaction of week × temperature × population indicated the germination responses to temperature over time were not the same for all three regional populations (table 3).

Initial germination (week-4) of seeds from the southern region was higher (49-65%) for cooler temperatures (27/15, 29/19, 22/11°C) than for the summer temperature (33/2°C) and MAS (3-6%), however by week-52, germination was similar (52-90%) at all temperatures tested (figure 6). Most germination had occurred by week-12 except at 33/24°C and in the MAS, which continued to increase until week-20. Initial germination for seeds from the southcentral region was similar across all temperatures at week-4 (1-31%) and maximum germination was achieved for most temperatures by week-12.
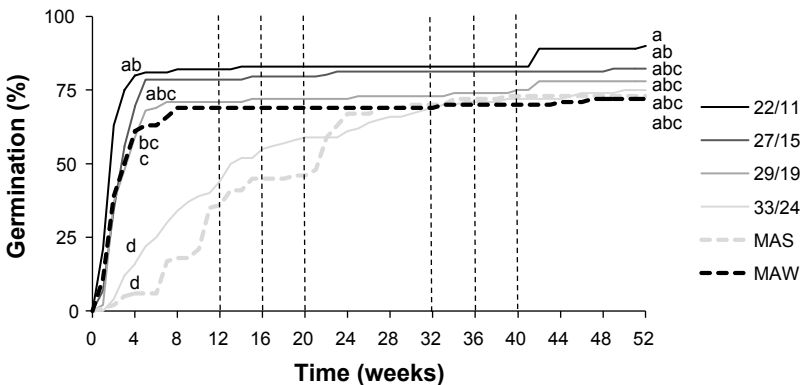

Figure 5. Germination of coastalplain honeycombhead (*Balduina angustifolia*) seeds collected from central Florida (Scrub Oak Preserve) in 2007. Seeds were placed in Petri dishes in a growth chamber with a 12-hour photoperiod for 52 weeks set at four seasonal constant temperatures [winter (22/11°C), early-spring or late-autumn (27/15°C), early-autumn or late-spring (29/19°C) and summer (33/24°C)] and two move-along temperatures that progress over time mimicking Florida seasonal temperature changes starting in summer (MAS) or winter (MAW). Seasonal and move-along temperatures are represented with solid and dotted lines, respectively. Vertical lines within graphs represent temperature changes in the MAS and MAW treatments. Letters above observations at week four and 52 represent differences in means determined using LSmeans (at α = 0.05).

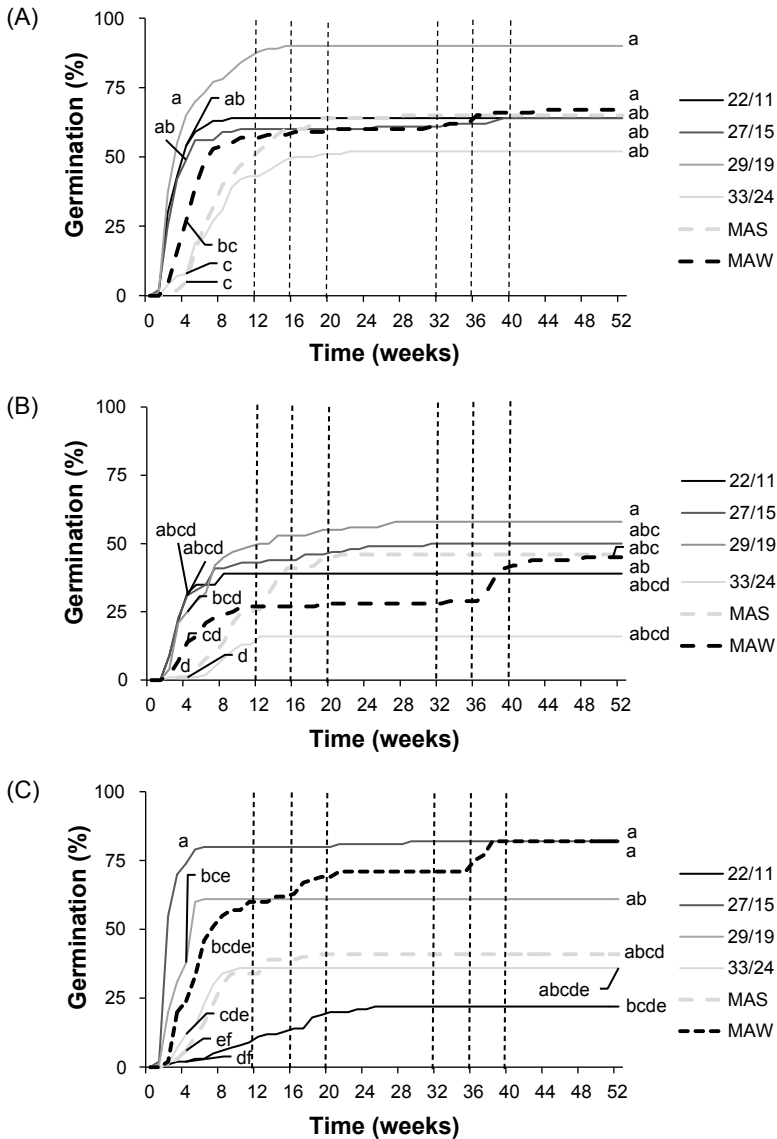Figure 6. Germination of *Balduina angustifolia* (coastalplain honeycombhead) seeds from three populations in south (**A**), southcentral (**B**) and northwest (**C**) Florida. Seeds from south, southcentral and northwest Florida populations were collected from Archbold Biological Station, Bok Tower Gardens and Navarre Beach in the autumn (late- November to early-December) of 2018. Seeds were placed in Petri dishes in a growth chamber with a 12-hour photoperiod for 52 weeks set at four seasonal constant temperatures [winter (22/11°C), early-spring or late-autumn (27/15°C), early-autumn or late-spring (29/19°C) and summer (33/24°C)] and two move-along temperatures that progress over time mimicking Florida seasonal temperature changes starting in summer (MAS) or winter (MAW). Seasonal and move-along temperatures are represented with solid and dotted lines, respectively. Vertical lines within graphs represent temperature changes in the MAS and MAW treatments. Letters above observations at week-4 and -52 represent differences in means determined using LSmeans (at α = 0.05) within each population (week × population × temperature; $P = 0.0311$).

Notable increases after week-12 were for MAS between weeks-12 and -20 and for MAW between weeks-32 and -52. Although germination increased over time germination remained similar across all temperatures (34-68%) by week-52.

Initial germination of seeds from the northwest region was higher (74%) at 27/15°C at week-4 compared with all other temperatures (2-38%) (figure 6). Most germination was complete by week-12 except for seeds in the MAW, MAS or at 22/11°C. Seeds in the MAS and at 22/11°C were nearly at their maximum germination by week-20 while seeds in the MAW did not reach maximum germination until week-38. By week-52, germination did not differ among the temperature treatments except that germination for MAW and the early-spring or late-autumn (27/15°C) temperature was greater than germination in winter temperatures (22/11°C) (82 vs. 22%) (figure 6).

Gibberellic acid (northwest Florida)
Viability of fresh seeds (five days after collection) from the northwest Florida populations of Deer Lake State Park, Navarre Beach, Big Lagoon State Park and Perdido Key was 77, 76, 74 and 84%, respectively. Germination of fresh seeds was influenced by both GA and population, and the significant ($P < 0.0001$) interaction of GA × population indicated the response to GA was not the same for seeds from the different northwest Florida populations (table 3). Seeds of Deer Lake State Park and Navarre Beach had similar germination with or without GA application (75-87 and 57-67%, respectively) (figure 7). Non-treated seeds from the two westernmost populations had minimal germination. GA application at 250 and 500 ppm for Big Lagoon State Park seeds resulted in higher germination (19-31%) compared with 0 ppm (1%) and there was no difference in germination between 125 ppm and 250 ppm (14-19%). Similarly, GA application at 250 and 500 ppm for Perdido Key
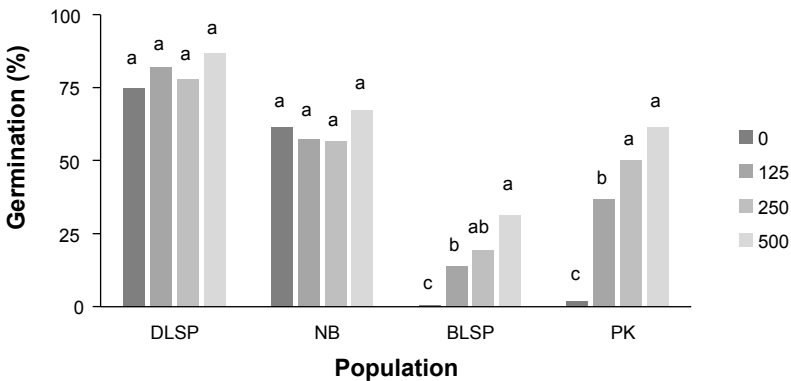

Figure 7. Effects of gibberellic acid (0, 125, 250 and 500 ppm; see legend) on the germination of seeds collected from four populations of *Balduina angustifolia* (coastalplain honeycombhead) collected five days (12/12/2018) prior to experimental initiation. Populations are as follows: DLSP = Deer Lake State Park, NB = Navarre Beach, BLSP = Big Lagoon State Park, PK = Perdido Key. Germination was calculated as (number of seeds germinated / (total number of seeds − diseased seeds)). Seeds were placed in a growth chamber set at 29/19°C day/night temperatures with a 12-hour day photoperiod of cool-white fluorescent light (76 ± 7.5 μmol m^{-2} second^{-1}). Letters above bars represent differences in means determined using LSmeans (at $\alpha = 0.05$) within each population (gibberellic acid × population; $P < 0.0001$).

seeds resulted in the highest germination (50-61%) compared with 250 ppm (37%) and the lowest germination occurred with 0 ppm GA (2%).

Germination of stored seeds was influenced by both storage and GA treatment and the response was different across populations (table 3). Deer Lake State Park was the only population with a decrease in gemination of nontreated seeds after storage (figures 7 and 8). All other populations show an increase in germination for non-treated seeds after storage (figure 8). Germination of non-treated seeds from both Big Lagoon State Park (27%) and Perdido Key (44%) after dry storage had germination percentages similar to seeds from the same lots treated with GA when fresh (figures 7 and 8). GA treatment at 500 ppm resulted in similar germination (78-92%) among all four seed lots after dry storage and only the Navarre Beach population had similar germination for both non-treated (88%) and GA-treated (90%) seeds. Disease was ≤ 11% across all populations.
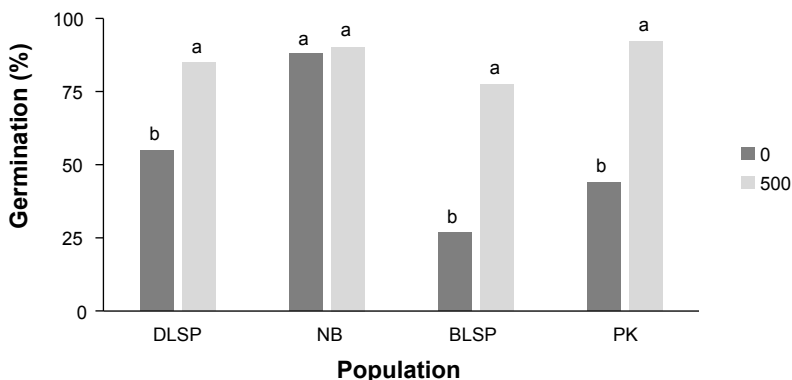

Figure 8. Effects of gibberellic acid (0 or 500 ppm) on the germination of seeds collected from four northwest Florida panhandle populations of *Balduina angustifolia* (coastalplain honeycombhead) after one year in storage under ambient laboratory conditions (approximately 23°C). Populations are as follows: DLSP = Deer Lake State Park, NB = Navarre Beach, BLSP = Big Lagoon State Park, PK = Perdido Key. Germination was calculated as (number of seeds germinated / (total number of seeds – diseased seeds)). Seeds were placed in a growth chamber set at 29/19°C day/night temperatures with a 12-hour day photoperiod of cool-white fluorescent light (76 ± 7.5 $\mu mol\ m^{-2}\ second^{-1}$). Letters above bars represent differences in means determined using LSmeans (at $\alpha = 0.05$) within each population (gibberellic acid × population; $P < 0.0001$).

Discussion

Seeds of honeycombhead will germinate under light or dark conditions (figure 3) indicating seeds can germinate on the surface of sandy soils or after sand burial characteristic of the coastal backdune and scrub communities where it occurs (Miller *et al*., 2001; Petrů and Menges, 2004). This is further supported by a study conducted by Petrů and Menges (2004) on seeds from scrub in south-central Florida where similar germination (15-20%) occurred in light (0 mm sand burial) and dark (5 mm sand burial). However, seeds from the same study had < 2% germination with 20 mm of sand burial. In coastal areas of the Florida panhandle, honeycombhead is restricted to the backside of foredunes, coastal grasslands

and stable areas of backdunes inland of foredunes (Davis, 2020). These backdune areas have more stable soils and less sand accretion compared with foredunes, indicating sand burial could contribute to the zonation of honeycombhead populations within the coastal landscape. As such, we recommend limiting the sowing of seeds of honeycombhead to apparently stable areas of coastal backdunes with minimal sand accretion.

While honeycombhead seeds collected from across the state and over multiple years had high gemination under moderate temperatures (spring and autumn temperatures), the germination response to more extreme temperatures (winter and summer) varies across its range (figures 3, 5 and 6). This difference in germination behaviour may, in part, explain the differences in life cycles reported within the state (Parker and Jones, 1975; Anderson and Menges, 1997). While we recognise the potential issues with comparing the data from germination tests conducted over different years, there is a still a general trend that emerges within the data. In south to central portions of its range, plants are reported as biennials which initially grow as a rosette prior to producing aerial stems with flowers. Plants then die during the second year (Anderson, 1975). In the northwest portion of its range, field observations over multiple years confirm the consistent annual nature (Thetford, *pers. obs.*; Davis *et al.*, 2020). Seeds from populations in south to central Florida exhibited high germination in winter temperatures and low germination in summer temperatures, whereas the opposite was true for northwest populations which had high germination in summer temperatures and low germination in winter temperatures. Seeds from the central to southern portions of honeycombhead's range can germinate under winter conditions present at dispersal during autumn and winter. Warmer winter temperatures of central and south Florida (figure 1) are sufficient to support germination and initial shoot growth the same season of seed dissemination. Most stem elongation occurs in the spring and summer with flowering and fruiting following in the autumn and winter. Hence, each autumn or winter, a portion of honeycombhead plants within a patch are young seedlings appearing as rosettes while others are mature and flowering. In the absence of killing cold winter temperatures honeycombhead complete their life cycle the second autumn or winter following germination. In contrast, seeds from northwest Florida do not germinate until the spring after seed dispersal. This delay in germination is controlled by reduced germination under winter temperatures and in some populations (but not all), physiological dormancy. Seeds of plants in the northwest portion of honeycombhead's range germinate under spring conditions, flower in the autumn of the same year, and naturally senesce following seed maturation, thereby completing their life cycle in a single growing season. This allows young seedlings to avoid cold winter temperatures that kill honeycombhead (Thetford, *pers. obs.*) which are more likely in the northwest portion of the plants range compared with the central to south regions (figure 1).

Genetic and environmental conditions experienced by the mother plant play key roles in determining seed dormancy and germination characteristics of seeds (Baskin and Baskin, 2014). Honeycombhead seeds collected from different populations collected on a single day in the northwest Florida panhandle had either non-deep physiological dormancy or were dormant when fresh or stored for one year (figures 7 and 8). This indicates, in part, population level differences of germination and dormancy for honeycombhead like other species in the sunflower family (Imbert *et al.*, 1996; Jorritsma-Wienk *et al.*,

2007; Sun *et al.*, 2009) and species of other plant families (Keith and Myerscought, 2016; Siles *et al.*, 2016; Pérez and Kane, 2017; Kaye *et al.*, 2018). With differing dormancy responses evident among seed sources representing eastern and western populations across northwest Florida, more research is needed to better understand the factors that contribute to dormancy in honeycombhead. Given the patchy nature of this plant, and the highly fragmented landscapes in which they occur, it is likely that both genetic and environmental factors are at play, driving the differences noted here.

Time in storage (one year) changed the dormancy characteristics of the seeds compared with fresh seeds for the populations tested, but the change observed depended on the source population of the seeds (figures 7 and 8). The Deer Lake State Park seeds represented the easternmost population from the panhandle and were non-dormant when fresh, but after one year storage, GA improved germination, indicating a trend towards non-deep physiological dormancy for this population. The opposite was true for seeds of the westernmost panhandle populations: seeds of Big Lagoon State Park and Perdido Key were initially dormant ($\leq 2\%$ vs. 31-61% germination for 0 vs. 500 ppm GA) but had improved germination (27-44%) for seeds with 0 ppm GA after one year in storage, indicating a trend towards non-dormancy for these populations. Seeds from Navarre Beach were from a central panhandle population and were nondormant when fresh as seen with seeds from Deer Lake State Park. Seeds from Navarre Beach also had similar germination across years and GA treatments indicating non-dormancy as seen with seeds of Big Lagoon State Park and Perdido Key.

Seeds of honeycombhead are orthodox and can be stored for over a year without specialised conditions and retain high viability. Seeds germinate easily without the need for specialised methods. Plants displayed variability at both a local level and regional level regarding reproductive characteristics, seed dormancy (non-dormant and non-deep physiological dormancy), and germination requirements. Given this information, restoration and conservation work involving honeycombhead should consider using hyperlocal seeds whenever possible.

The recommendation for localised seed use is supported, in part, by the association of this plant with the endemic coastal plain hesperapis. The presently known range of the coastal plain hesperapis in Florida is restricted to coastal mainland and barrier islands of the Florida panhandle which includes the panhandle sites where seeds for this work were collected. Given the geographical differences in germination response noted between the regional populations of honeycombhead and the differences in dormancy noted in the narrow range of panhandle populations, consideration should be given to restricting restoration planting of honeycombhead associated with the coastal plain hesperapis to panhandle populations.

Acknowledgements

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