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NOTE

Variables influencing germination and initial survival of two critically endangered plants: *Warea amplexifolia* and *Lupinus aridorum*[1]

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Abstract: One challenge of ex situ conservation is developing propagation methods that promote a high rate of survival and genetic diversity. Developing successful propagation methods is especially important for rare species to prevent their extinction. Clasping warea, *Warea amplexifolia* (Nutt.) Nutt. (Brassicaceae), and scrub lupine, *Lupinus aridorum* McFarlin ex Beckner (Fabaceae), are two rare species endemic to imperiled Florida sandhill and scrub habitats, respectively. We tested whether the collection site of seeds, seed stratification temperature, and several propagation methods influenced germination and initial survival of *W. amplexifolia* and *L. aridorum*. The collection site of seeds and type of pot influenced percent germination of *W. amplexifolia*, whereas soaking seeds in water and stratification temperature did not. The site where seeds were collected did not influence germination of *L. aridorum* and treating seedlings with salicylic acid, nitrogen, or salicylic acid and nitrogen sometimes reduced, but did not increase, initial survival of seedlings. Overall, our results will inform additional experiments on ex situ conservation and may be applicable to other herbs endemic to Florida.

Key words: clasping warea, scrub lupine, Florida scrub, sandhill, seed origin, ex situ conservation.

Résumé : Un des défis de la conservation ex situ consiste à développer des méthodes de propagation favorisant un taux de survie élevé et une diversité génétique. La mise au point de méthodes de propagation efficaces revêt une importance particulière pour les espèces rares, afin de prévenir leur extinction. Le *Warea amplexifolia* (Nutt.) Nutt. (Brassicaceae) et le *Lupinus aridorum* McFarlin ex Beckner (Fabaceae), représentent deux espèces rares endémiques des habitats menacés de dunes et de broussailles en Floride, respectivement. Les auteurs ont examiné si le site de collection des semences, la température de stratification des graines et plusieurs méthodes de propagation influencent la germination et la survie initiale du *W. amplexifolia* et du *L. aridorum*. Le site de récolte des graines et le type de pot influencent le pourcentage de germination du *W. amplexifolia,* alors que le trempage des graines dans l'eau et la stratification restent sans effet. Le site de récolte des graines n'influence pas la germination du *L. aridorum* et le traitement des graines à l'acide salicylique, à l'azote, ou à l'acide salicylique et azote diminuent quelques fois, mais n'augmentent pas la survie initiale des plantules. Dans l'ensemble, les résultats obtenus pourront servir à d'autres expériences sur la conservation ex situ et pourraient être applicables à d'autres herbacées endémique de la Floride. [Traduit par la Rédaction]

Mots-clés : Warea amplexifolia, *Lupinus aridorum*, broussailles de la Floride, dunes de sable, origine des semences, conservation ex situ.

Introduction

One of the initial challenges in ex situ conservation is developing propagation methods that result in a high rate of survival. Developing successful propagation methods is especially important for rare species because their remaining genetic diversity needs to be preserved to prevent extinction of the species. Clasping warea, *Warea amplexifolia* (Nutt.) Nutt. (Brassicaceae), and scrub lupine, *Lupinus aridorum* McFarlin ex Beckner (Fabaceae), are two of Florida's most critically endangered plant species and require ex situ conservation. *Warea amplexifolia* and *L. aridorum* are endemic to Florida sandhill and scrub habitats, respectively. Sandhill and scrub support many of the state's rare species because these two habitats are imperiled and have been reduced by at least 88% and 66%, respectively, due to anthropogenic activities (Ross 1995; Kautz 1998). Very little is known about the biology and ecology of *W. amplexifolia* and *L. aridorum*. *Warea amplexifolia* blooms from August through October and is restricted to Candler soil in Marion, Polk, and Lake Counties. *Warea amplexifolia* can only reproduce sexually, produces fruit in late September through December when the plants senesce, and overwinters as seed. The species is somewhat unique within sandhill habitat because it is an annual, whereas most species are perennials (Weekley et al. 2007). Annual life cycles are often adapted to habitats where the likelihood that a seed will become a flowering plant in 1 year is greater than the likelihood that an adult plant will survive for multiple flowering seasons (Watkinson and Davy 1985). However, being restricted in range and numbers makes *W. amplexifolia* extremely vulnerable to disturbance and natural disasters. Any disturbance that reduces or eliminates reproduction or the overwintering seed bank could cause extirpation of a population and loss of genetic variability in the species.

Lupinus aridorum is a short-lived perennial herb that was first described in 1982 (Beckner 1982). It blooms from March through May, fruits in June, and is restricted primarily to St. Lucie soil in Orange and Polk counties. Most of the remaining plants are on private property that has been highly modified or is undergoing development. For example, a population in Orange County is located in highly modified habitat between the city of Orlando and Walt Disney World.

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The Center for Plant Conservation, a national organization of gardens and other institutions in the United States (Shaw 1985), strives to preserve germplasm of rare plants and uses the germplasm to cultivate plants. The Rare Plant Conservation Program at Bok Tower Gardens (BTG; Lake Wales, Florida, USA), a member of the Center for Plant Conservation, cultivates rare plant species in Florida, including *W. amplexifolia* and *L. aridorum*, and reintroduces them into their native habitats (Bissett 1987). *Warea amplexifolia* has been introduced into two sites beginning in 2011 and *L. aridorum* has been introduced into six sites beginning in 2008. The plants used in these introductions were propagated in a greenhouse at BTG, which was made possible by developing propagation methods between 1986 and 2011 that influenced germination and early survival of these species. Variables tested for their influence on germination and early survival include light intensity, collection site of seeds, abiotic factors of the collection site (type of soil, pH, macronutrients), various seed treatments (smoke, ash, pre-soaking in water, plant growth regulators, storage temperatures, stratification, and scarification), seed age at sowing, container type (pots and plug trays), plant hormones, and fertilization. None of the propagation experiments have been published, so we present data from our recent experiments, in which we tested the influence of seed collection site, seed soaking, pot type, and stratification temperature on germination of *W. amplexifolia*. We also tested whether the collection site of seeds influenced germination of *L. aridorum* and whether treating plants of this species with salicylic acid and (or) nitrogen to reduce physiological stress improved their early survival. The propagation methods largely differed between the two species because our previous, unpublished work indicated that they have different requirements for germination and that *L. aridorum* experiences higher levels of stress during early survival than *W. amplexifolia*. We chose these particular propagation methods because they were previously not tested for these species or because previous trials indicated that they were likely to have important effects on germination and survival. In addition, most of these variables influence germination of other plant species (Baskin and Baskin 1998).

Materials and methods

Influence of collection site, seed stratification, and propagation methods on germination of *Warea amplexifolia*

We collected seeds from *W. amplexifolia* in two sites (Polk and Lake Counties) during fall and winter 2010, approximately 3 months prior to planting. Storing seeds for 3 months does not influence rates of germination (unpublished data). The two sites are separated by greater than 97 km, so gene flow between the plant populations is unlikely. Seeds from Lake County were stored at ambient temperatures (23.5 °C) in the office of the Rare Plant Curator at BTG prior to planting. Seeds from Polk County were divided into three groups and each group was subjected to one of the following stratification temperatures: ambient temperature; refrigerated (7 °C); or frozen (–20 °C). Seeds were held at the latter two storage temperatures for approximately 2 weeks and then gradually brought to ambient temperature over 2–3 days, so we could test the influence of cold stratification on germination. Winters in central Florida typically consist of short periods of cold weather, so this duration of stratification likely mimics natural conditions. Approximately half the seeds from each collection site were then soaked in tap water for 24 h prior to planting, whereas the other half were not. We planted 10–15 seeds per treatment and replication in 6.25 cm peat or plastic pots containing a 3:1 ratio of native soil and Fafard 2 Mix (Conrad Fafard Inc., Agawam, Massachusetts, USA). Pots were then placed in a greenhouse with retractable walls to allow natural ventilation (Nexus, Inc., Northglen, Colorado, USA), and we counted the number of seeds that germinated in each treatment and replication within 3 months of the planting date. See Table 1 for the number of replications for each treatment.

Table 1. Mean percent germination (±SEM) of seeds from *Warea amplexifolia* collected from two sites and subjected to three propagation methods.

Term	Variable	*N*	Mean (±SEM)*
Collection site	Polk County	48	49.0 (3.1)a
	Lake County	16	29.4 (3.2)b
Pot type	Plastic	38	50.8 (3.1)a
	Peat	26	34.2 (4.1)b
Seed soaking	Soaked	31	45.8 (3.9)
	Not soaked	33	42.4 (3.7)
Stratification temperature	Ambient	31	40.4 (3.8)
	Refrigerated	16	41.7 (4.4)
	Frozen	17	52.9 (5.7)

*Means followed by different letters within a term are significantly different (means separation test, $P < 0.05$).

We determined the influence of seed collection site (Polk or Lake County), seed soaking (soaked in water or not), pot type (peat or plastic), and stratification temperature (ambient, refrigerated, or frozen) on percent germination with ANOVA (PROC GLM, SAS Institute 2011). We originally included interactions between the independent variables in our model, but removed them because they were not significant (Sokal and Rohlf 1995). The LSMEANS statement was then used to estimate separation between pairs of means (SAS Institute 2011; Sokal and Rohlf 1995).

Germination of seeds from Lake County was low in the prior experiment (see Results), but we wanted to retain the genetic diversity of plants from this population, so we used seeds collected from this location in December 2010 to determine whether we could identify the planting date that optimized germination. Seeds were stored at ambient temperatures in the office of the Rare Plant Curator at BTG and then frozen for 2 weeks at BTG prior to planting. Seeds were planted in a mixture of native soil and Fafard 2 Mix in 5 cm plastic pots and placed in a greenhouse, as previously discussed. We planted seeds 5 March (n = 4 replications), 13 March (n = 30), 14 March (n = 32), and 6 April 2012 (n = 16), which is within the range of when plants germinate naturally in wild populations. Each pot, which is a replication, was planted with 5–10 seeds. The number of seeds that germinated in each replication was counted 1 month after the final planting date. We used ANOVA with means separation, as previously discussed, to investigate the influence of planting date on percent germination.

Influence of collection site and seedling treatments on germination and initial survival, respectively, of *Lupinus aridorum*

We collected seeds from *L. aridorum* at four sites, three in Orange County and one in Polk County, in June 2010 for planting in September 2010 to test the influence of seed collection site on germination. Seeds were stored at ambient temperatures in the office of the Rare Plant Curator at BTG after harvest. Seeds were scarified using 100 grit sandpaper until scratch marks were visible, but no color change was noted and the seed coat was not cracked. We soaked seeds in tap water for 2 h, rolled them in a 1:1 mix of powdered rooting hormone (Schultz® TakeRoot® Rooting Hormone, Schultz Company, Bridgeton, Missouri, USA) and mycorrhizal symbionts (Diehard Starter Mix, Horticultural Alliance, Inc., Sarasota, Florida, USA), and planted them individually in cells of a 288-count plug tray with a 1:1 mix of native soil and Fafard 2 Mix. *Lupinus aridorum* seeds were rolled in mycorrhizal symbionts to reduce stress from subsequent repotting and transplanting. Each site was replicated with two to four trays and contained a mean of 186 seeds within each replication. The number of seeds that germinated in each tray was counted within 3 months after planting. We repeated this experiment in 2011 with seeds collected from the following three populations: one in Polk County and two in Orange County. We used separate ANOVAs with means separation, as previously discussed, to investigate the influence of collection site on percent germination of seeds in 2010 and 2011.

We also tested the influence of nitrogen and salicylic acid on survival of approximately 3-week-old seedlings of *L. aridorum*. Four hundred seeds were collected from the Endangered Plant Garden at BTG in 2009 and prepared and planted 18 November 2010 as described for the previous experiment. We assigned 100 seeds each to one of the following four treatments: control (distilled water), salicylic acid, nitrogen, or salicylic acid and nitrogen. Salicylic acid is a plant hormone that is involved in plant growth, metabolism, and other physiological processes and may induce tolerance to stress (Senaratna et al. 2000; Hayat and Ahmad 2007). We used 0.1 mmol/L of salicylic acid in 1 L of distilled water to test whether it would improve survival of plants shortly after germination when they were transplanted from trays into 10 cm pots. However, salicylic acid can inhibit nitrogen uptake by some species of legumes (Billou et al. 1999; Stacey et al. 2006), so we also used 2 mmol/L of nitrogen, individually in 1 L of distilled water and mixed with 0.1 mmol/L of salicylic acid in 1 L of water, to test whether the addition of nitrogen mitigated any loss of nitrogen uptake caused by application of salicylic acid. Each plant was drenched with 94 mL of total solution immediately after transplanting them into 10 cm pots on 20 December 2010 and survival was noted through 31 January 2011. We determined the influence of treatment on survival using a binomial regression model (PROC GENMOD, SAS Institute 2011). We then used the LSMEANS statement to estimate separation between pairs of means (SAS Institute 2011; Sokal and Rohlf 1995). Sample size was reduced to 55–73 individual plants per treatment because some seeds failed to germinate or seedlings died before treatment.

Results

Influence of collection site, seed stratification, and propagation methods on germination of *Warea amplexifolia*

The collection site of seeds and the type of pot influenced percent germination of *W. amplexifolia*, whereas soaking seeds and stratification temperature did not (overall ANOVA, $F_{[5,58]} = 5.6$, $P < 0.001$; collection site, $F_{[1]} = 10.7$, $P = 0.002$; pot type, $F_{[1]} = 10.1$, $P = 0.002$; seed soaking, $F_{[1]} = 0.42$, $P = 0.52$; stratification temperature, $F_{[2]} = 1.7$, $P = 0.19$). Percent germination was ~1.7 times higher for seeds from Polk County than Lake County and ~1.5 times higher for seeds in plastic pots versus peat pots (Table 1). The planting date also highly influenced percent germination ($F_{[3,78]} = 11.0$, $P < 0.001$): percent germination was ~1.7–2.6 times higher for seeds that were planted in mid-March compared to early March or April (Fig. 1).

Influence of collection site and seedling treatments on germination and initial survival, respectively, of *Lupinus aridorum*

The site where seeds were collected did not influence percent germination of *L. aridorum* in 2010 ($F_{[3,8]} = 3.7$, $P = 0.06$) or 2011 ($F_{[2,6]} = 2.0$, $P = 0.22$). The treatment of seedlings with salicylic acid, nitrogen, or salicylic acid and nitrogen influenced survival of seedlings ($\chi^2 = 17.8$, df = 3, $P < 0.001$). However, none of the treatments had higher survival than the control and the mean percentage of seedlings that survived in the control was nearly double the survival of seedlings treated with nitrogen, which caused high mortality (Fig. 2).

Discussion

The site where seeds were collected influenced germination of *W. amplexifolia*, but not *L. aridorum*. We are unsure whether this difference is due to the annual versus perennial life cycles of these plants, genetics (perhaps populations of *L. aridorum* are more genetically similar than populations of *W. amplexifolia*), or some other factor. Nonetheless, where seeds are collected can have an important influence on germination of some plants species, which in turn may influence the genetic diversity of reintroduced populations of rare plants. Genetic diversity needs to be maximized in introduced and augmented populations to avoid genetic bottlenecks, inbreeding depression, and poor establishment of plants (Vander Mijnsbrugge et al. 2010). In the case of *W. amplexifolia*, genetic factors may result in the differential rates of germination between seeds collected at the two locations. The site with the higher germination rate (Polk County) had a population size of only 10 plants in 2010 and had not been found during surveys at that location since 1998; the population likely persisted in the seed bank. The site with the lower rate (Lake County) contained over 1000 plants in 2010. Therefore, the surviving plants in Polk County may have wider tolerance to environmental conditions and higher fitness than the average plant in Lake County.

We found that the type of pot and timing of planting can also influence germination. Plants may have higher germination rates in plastic pots because these pots retain soil moisture longer than peat pots, which may be especially important for plant species

Fig. 1. Mean percent (±SEM) germination of *Warea amplexifolia* seeds planted on four dates. Means with different letters are significantly different (means separation test, $P < 0.05$).

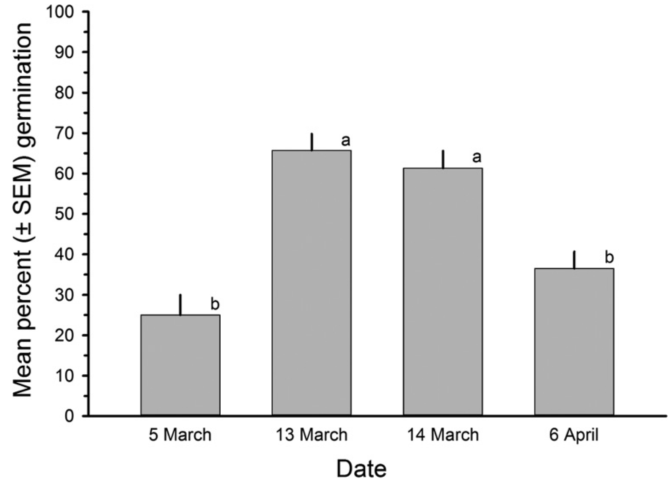
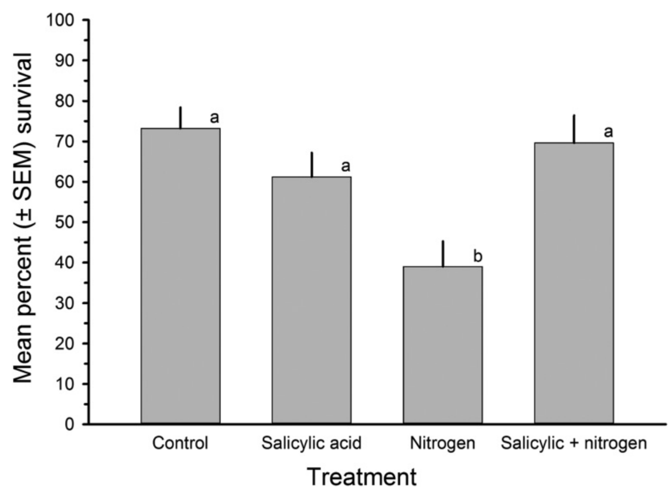


Fig. 2. Mean percent (±SEM) survival of *Lupinus aridorum* seedlings that were subjected to four treatments. Means with different letters are significantly different (means separation test, $P < 0.05$).



that grow in sandy soil that dries quickly, such as *W. amplexifolia*. We are unsure why rates of germination varied over a relatively small range of planting dates, but apparently rates of germination are influenced by ambient sunlight and heat and are highest during a very narrow period of time. Overall, our results suggest that optimizing propagation techniques may help increase germination and may especially be beneficial for increasing rates of germination in populations of rare plants that naturally have lower rates of germination.

Lupinus aridorum is highly susceptible to stress and experiences high mortality when its roots are disturbed during repotting or transplanting in the field. We added nitrogen and salicylic acid alone, and in combination, in an attempt to reduce stress and increase survival. However, nitrogen and salicylic acid did not increase survival rates of seedling *L. aridorum*. In fact, nitrogen decreased survival of seedlings and we are unsure why nitrogen would have this effect.

In conclusion, our results will inform additional experiments on ex situ conservation and reintroduction of *W. amplexifolia* and *L. aridorum* as well as related species. Many species within the Brassicaceae and Fabaceae, including three additional species of *Warea* and eight additional *Lupinus* species, are endemic to Florida and our propagation techniques could likely be used to guide their conservation. Our results also are likely applicable to other short-lived herbaceous species in Florida scrub and sandhill because they are narrowly adapted only to the disturbance regime and microhabitat specific to this imperiled habitat (Hartnett and Richardson 1989; Menges et al. 1999; Richardson et al. 2013).

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