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Author(s): Matthew A. Albrecht and Juan Carlos Penagos Z.
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Seed germination ecology of three imperiled plants of rock outcrops in the southeastern United States[1,2]

Matthew A. Albrecht[3] **and Juan Carlos Penagos Z.**

Center for Conservation and Sustainable Development, Missouri Botanical Garden, PO Box 299,
St. Louis, MO 63166

ALBRECHT, M. A. AND J. C. PENAGOS Z. (Center for Conservation and Sustainable Development, Missouri Botanical Garden, PO Box 299, St. Louis, MO 63166). Seed germination ecology of three imperiled plants of rock outcrops in the southeastern United States. J. Torrey Bot. Soc. 139: 86–95. 2012.—The success of rare plant conservation programs depends upon understanding the ecological factors that regulate seed dormancy and germination. In this study, we characterize the germination niche with respect to temperature and light of three imperiled perennials that are endemic to rock outcrops in the southeastern United States: *Astragalus bibullatus*, *Claytonia ozarkensis*, and *Conradina verticillata*. Our results show that a majority of seeds for each species are unable to germinate at habitat temperatures prevailing after dispersal in early summer. Seeds of the rockface endemic *Claytonia ozarkensis* germinated to high rates in darkness at 5°C, suggesting germination is confined to winter and that seeds are unable to persist beyond one germination season (transient seed bank). For *Conradina verticillata*, 44% of seeds germinated without cold stratification, while the remainder required cold stratification and light to overcome physiological dormancy. Following cold stratification, *Conradina verticillata* seeds germinated in light at cool (15/5 °C) but not at warm (30/15 °C) temperatures, although overall seed viability was low (26%). Seeds of *Astragalus bibullatus* germinated to low rates ($< 10\%$) in seasonal temperature sequences, confirming that this species forms a persistent seed bank. Seeds of *Astragalus bibullatus* lost viability following heat shock for 30 min at 125 °C, but germinated to $> 50\%$ following mechanical scarification and incubation at 30/15 °C. Results from this study can be used to maximize germination for *ex situ* and reintroduction programs, and provide insight into managing wild populations.

Key words: *Astragalus*, *Claytonia*, *Conradina*, *ex situ* conservation, germination niche, rare plants, seed dormancy.

Seed germination is a critical stage in the life cycle of plants and is restricted in space and time to locations that meet a specific set of environmental conditions (‘safe sites,’ Harper 1977). In temperate habitats, seeds of many species are dormant at maturity and are unable to germinate at prevailing habitat temperatures following dispersal, which are typically unfavorable for seedling establishment (Vleeshouwers et al. 1995). Temperature is the primary factor regulating dormancy break and germination timing, with seeds of temperate herbaceous perennials most frequently germinating in autumn or spring after fulfilling a warm and/or cold temperature requirement for dormancy break (Baskin and Baskin 2001, Walck et al. 2005). Once dormancy is lost, nondormant seeds may require specific temperatures and environmental cues (e.g., light) to germinate (Vleeshouwers et al. 1995, Baskin and Baskin 2001); otherwise seeds can become conditionally dormant and enter the soil seed bank. While members of certain plant families can exhibit similar types of dormancy (e.g., physical dormancy, Fabaceae), species that occupy similar habitats often exhibit different dormancy and germination traits due to variation in seed coat permeability, life history, phylogeny, and response to environmental cues (Grime et al. 1981, Baskin and Baskin 2001, Vandelook et al. 2008).

Understanding factors that regulate seed dormancy and germination in rare plant species is essential for integrated conservation strategies, which combine *in situ* habitat protection and management with safeguarding genetic material in *ex situ* seed banks

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[3] Author for correspondence. E-mail: matthew.albrecht@mobot.org

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(Maunder et al. 2004, Havens et al. 2006). *Ex situ* collections serve as the source material for the reintroduction of rare plants into areas where a species has gone extinct or to bolster the size and improve reproductive output of small populations (Guerrant and Pavlik 1998, Cochrane et al. 2007, Maschinski and Duquesnel 2007). Successful conservation of a species hinges not only on the quality of these *ex situ* collections but also on developing protocols to overcome seed dormancy (Merritt et al. 2007, Wall et al. 2010), which can vary widely among species and seed dormancy types. Indeed, knowledge gaps on dormancy breaking and germination protocols for rare plant species have been cited as a major obstacle in understanding the viability of *ex situ* collections (Godefroid et al. 2010), developing autecological research programs to support species conservation efforts (Trusty et al. 2009), and producing sufficient quantities of plant material for restoration or reintroduction (Merritt et al. 2007). Species-specific experiments are necessary to understand the environmental controls over seed germination because germination niches can vary widely between congeneric species (Grime et al. 1981, Young et al. 2007, Vandelook et al. 2008).

Rock outcrops and other shallow-soil plant communities, including river scour prairies and rockfaces (hereafter collectively referred to as rock outcrops), are patchily distributed throughout forested regions of the southeastern US (Braun 1950, Anderson et al. 1999). A notable feature of rock outcrops is the distinctive flora that includes a large number of rare endemic plant species, many of which are now imperiled due to restricted distributions, low population numbers, and widespread habitat loss and degradation (Baskin and Baskin 1988, Anderson et al. 1999). Three examples of imperiled rock outcrop endemics in the southeastern US include *Astragalus bibullatus* Barneby & Bridges (Fabaceae), *Claytonia ozarkensis* Miller & Chambers (Portulacaceae), and *Conradina verticillata* Jennison (Lamiaceae), which are the focus of this study. Due to their global rarity and high level of endangerment in the wild, all three focal species are maintained in *ex situ* collections (primarily as seeds) as part of the Center for Plant Conservation's (CPC) National Collection of Endangered Plants. Yet little is known about the germination niche of each species, limiting the effective utilization of their *ex situ* collections for conservation and understanding these species' population dynamics in the wild.

When designed in an ecological context, *ex situ* germination studies can help characterize the germination niche of a rare species and the natural habitat conditions in which seeds are most likely to germinate. The goals of this study are to characterize the germination niche with respect to temperature and light of the rare rock outcrop endemics *Astragalus bibullatus*, *Claytonia ozarkensis*, and *Conradina verticillata.* Specifically, we asked the following questions: 1) How do seasonal temperatures influence germination timing? 2) Does light influence the probability of germination? and 3) Which regeneration traits (viability, dormancy, or germination) might limit seed regeneration in the wild?

STUDY SPECIES. *Astragalus bibullatus* is a federally endangered herbaceous perennial endemic to limestone cedar glades and known from eight extant occurrences in Rutherford County, Tennessee. Seeds of *A. bibullatus* are dispersed abiotically (gravity and water) and capable of long term persistence in the soil, reaching densities up to 750 m^{-2} (Morris et al. 2002). Soil seed populations contain greater levels of genetic diversity than aboveground populations (Morris et al. 2002), which exhibit little genetic differentiation according to studies using allozymes and neutral genetic markers (Baskauf and Snapp 1998, Baskauf and Burke 2009). Like most temperate members of the Fabaceae, seeds of *A. bibullatus* exhibit physical dormancy that can be broken artificially by scarification (McCue et al. 2001, Baskin and Baskin 2005), although little is known about the temperature requirements that might break seed dormancy in the wild. Efforts are currently underway to restore populations but are challenged by a paucity of information on seed germination (Albrecht and McCue 2010).

Known from three extant sites in Arkansas (and five historical sites in Missouri and Oklahoma), *Claytonia ozarkensis* is a recently described species restricted to crevices or fissures in moist sandstone rockfaces on wooded slopes (Miller and Chamberlain 2006). Little is known about the autecology of *C. ozarkensis.*

Conradina verticillata is a federally threatened perennial subshrub restricted to river scour prairies (a mixture of shallow soil and exposed bedrock, cobble, and/or gravel) on the Cumberland Plateau in northcentral Tennessee and southeastern Kentucky (USFWS 1996). Populations consist of distinct colonies that are numerically small and isolated from one another. The species reproduces via clonal reproduction and requires insects for viable seed production (USFWS 1996).

Methods. SEED COLLECTION. We followed the CPC's guidelines for germination experiments with rare plants by only using seeds collected from the largest and most secure population of each species (Guerrant et al. 2004). Seeds were collected for *Astragalus bibullatus* on 19 June 2009, *Claytonia ozarkensis* on 22 April 2009, and *Conradina verticillata* on 18 June 2009. After collection, seeds were air dried for ~15 d (*A. bibullatus* and *C. verticillata*) or ~35 d (*C. ozarkensis*) and stored at room temperature (~20 °C) until germination experiments commenced.

GERMINATION PROTOCOL. Because these imperiled species are known from few populations, the scope of our germination experiments was limited by seed availability.

For all experiments, we used five replicate Petri dishes per treatment. For *Astragalus bibullatus* and *Conradina verticillata*, ten seeds per replicate were used whereas 15 seeds per replicate were used for *Claytonia ozarkensis*. In all experiments, Petri dishes were filled with 40 g of sand and kept constantly moist by adding 8 mL of distilled water as needed. For dark treatments, Petri dishes were wrapped in two layers of aluminum foil and checked for germination when the treatment terminated (6 wks). For all light treatments, dishes were checked weekly and all germinants removed; radicle emergence > 1 mm was the criterion for germination. Seeds were placed in germination incubators set at a 12/12 h light/dark cycle daily with alternating thermoperiods of 15/5 °C and 30/15 °C. These thermoperiods approximate the mean daily maximum and minimum monthly air temperatures in the habitats of these species in March/April and November, 15/5 °C; and June–August, 30/15 °C (Hijmans et al. 2005). We chose a 12 hr daily photoperiod because a previous study with *A. bibullatus* reported relatively high germination rates at this photoperiod (Morris et al. 2002). Incubators were illuminated with cool white fluorescent bulbs at PAR (photosynthetically active radiation, 400–700 nm) of ~35 μmol m^{-2} at seed level. Petri dishes were randomized weekly in each incubator. All cold stratification treatments were conducted in darkness at 5 °C. At the end of each treatment, nongerminated seeds of *Claytonia ozarkensis* and *Conradina verticillata* were dissected to determine if seeds were filled with firm, white embryos. For conservation purposes, *A. bibullatus* seeds were not dissected to determine viability, which would have killed the embryo. Instead, nongerminated seeds that were firm when pinched with forceps were scored as viable.

EXPERIMENT 1. To determine if seeds of the focal species were dormant or nondormant at maturity, fresh seeds were placed in light and darkness at constant temperature regimes of 30/15 °C and 15/5 °C. Germination was checked weekly for seeds in the light treatment and at the end of 6 wks for seeds in the dark treatment. Seeds incubated in light at each temperature remained there for 56 wks and served as the controls for the cold stratification and move-along experiments (described below).

The effects of cold stratification were examined for *Claytonia ozarkensis* and *Conradina verticillata*. We suspected these two species exhibited physiological dormancy, which is the most common type of seed dormancy found in species of treeless habitats in the temperate deciduous forest region, and is typically broken by cold stratification (Baskin and Baskin 2001). We omitted *Astragalus bibullatus* seeds from this treatment due to limited seed availability. In a 2 × 2 factorial experiment, seeds of *Claytonia ozarkensis* and *Conradina verticillata* were cold stratified (in darkness) for 12 wk and then incubated for 6 wks in light and darkness at 15/5 °C and 30/15 °C. Seeds that were cold stratified and then incubated in the dark were not checked for germination until after 18 wk.

EXPERIMENT 2. For each species, we used move-along experiments to determine whether seeds required a cold, warm, or warm and cold stratification for dormancy break and germination (Baskin and Baskin 2004). Seeds were moved simultaneously through two different temperature sequences. The first (or warm) sequence mimics a summer → autumn → winter → spring temperature cycle: 30/15 °C

(12 wks) → 15/5 °C (6 wks) → 5 °C (12 wks) → 15/5 °C (6 wks) → 30/15 °C (12 wks) → 15/5 °C (6 wks) → 5 °C (12 wks). The second (or cold) sequence mimics a winter → spring → summer → autumn temperature cycle: 5 °C (12 wks) → 15/5 °C (6 wks) → 30/15 °C (12 wks) → 15/5 °C (6 wks) → 5 °C (12 wks) → 15/5 °C (6 wks) → 30/15 °C (12 wks).

EXPERIMENT 3. For *Astragalus bibullatus*, we conducted a 2 × 2 × 2 factorial experiment to compare germination fractions of seeds subjected to no pretreatment (control), heat, and scarification. For the heat treatment, twenty replicates of 10 seeds each were placed in a laboratory drying oven and heated at 125 °C for 30 min and then allowed to cool to room temperature after being removed from the oven. This heat treatment is within the range of soil temperatures that shallowly buried ($<$ 5 cm) seeds of *A. bibullatus* might experience during a prescribed fire (Trammell et al. 2004). For scarification treatments, seeds were scarified with sandpaper according to the methods in McCue et al. (2001). Replicates of the heat and scarification treatments were incubated in light and darkness at 30/15 °C and 15/5 °C for 4 wks. Controls consisted of Petri dishes of fresh seeds used in EXPERIMENT 1.

DATA ANALYSIS. For each experiment, germination fractions were calculated as the fraction of the number of seeds germinating in each dish to the total number of viable seeds in each dish at the end of each experiment. The fraction (mean ± SE) of viable seeds at the end of EXPERIMENTS 1 and 2 for *Astragalus bibullatus*, *Claytonia ozarkensis*, and *Conradina verticillata* were 0.89 ± 0.01, 0.72 ± 0.03, and 0.26 ± 0.04, respectively. For all statistical analyses, we analyzed germination fractions separately for each species' using Generalized Linear Models (GLM) with a logit link function and binomial error structure in the R statistical program version 2.6.2 (R Development Core Team 2008). We corrected for overdispersion using a quasibinomial error structure when necessary (Crawley 2007).

For EXPERIMENT 1, lack of germination in some treatment combinations precluded statistical testing of interactions between temperature, light, and treatments (stratified versus nonstratified). For *Conradina verticillata*, we tested the effects of light and temperature on germination only for seeds that were cold stratified because germination was negligible across most incubation temperature and light combinations in the controls. Since a majority of *Claytonia ozarkensis* seeds germinated while in cold stratification, sample sizes were too small to test for temperature and light effects on germination fractions (see Table 1). We used GLM a priori contrasts to test the hypothesis that seeds subjected to seasonal temperatures in EXPERIMENT 2 would exhibit greater germination fractions than seeds maintained at constant temperature regimes in EXPERIMENT 1.

For EXPERIMENT 3, we conducted two separate analyses since germination of *Astragalus bibullatus* seeds was negligible in the heat pretreatment and in the dark for control seeds. First, we tested whether germination fractions between the control and scarification differed between incubation temperatures. In the second analysis, we tested for the effect of incubation temperature and light only on the germination fractions of scarified seeds.

Results. EXPERIMENT 1. After 6 wks, no *Astragalus bibullatus* seeds germinated in the dark while $<$ 5% of seeds germinated in light at 30/15 °C and 15/5 °C (Table 1). Germination fractions for *A. bibullatus* were $<$ 10% after 18 wks in light. For seeds of *Claytonia ozarkensis* germination fractions were significantly greater (P = 0.01) in the dark than in light at 15/5 °C after 6 wks whereas no nonstratified seeds germinated in the dark or light after 18 wks at 30/15 °C (Table 1). For *Conradina verticillata,* no seeds germinated in darkness at either temperature regime after 6 wks. After 18 wks, *C. verticillata* seeds germinated in light at 30/15 °C whereas only 1 seed germinated in light at 15/5 °C (Table 1).

Cold stratification generally increased germination fractions of *Claytonia ozarkensis* and *Conradina verticillata* seeds relative to nonstratified seeds. However, cold stratification effects on germination fractions of *C. verticillata* seeds depended on light and incubation temperatures (light × temperature interaction: $P < 0.0001$). In light, germination fractions after cold stratification were significantly greater (Tukey test, $P < 0.01$) at 15/5 °C than at 30/15 °C, but there were no differences in germination fractions among incubation temperatures in the dark (Tukey test, $P = 0.86$, Table 1).

EXPERIMENT 2. In the move-along experiment, germination fractions were significantly

Table 1. Germination fractions (mean ± SE) of fresh, nonstratified, and stratified seeds of three imperiled plants. *Asbi* = *Astragalus bibullatus*, *Cloz* = *Claytonia ozarkensis*, *Cove* = *Conradina verticillata.* NT = Treatment not tested. Values in parentheses are germination fractions after 12 wks of cold stratification.

			30/15 °C		15/5 °C	
Species	Condition	Weeks	Light	Dark	Light	Dark
Asbi	Fresh	6	0.02 ± 0.02	0 ± 0	0.04 ± 0.02	0 ± 0
	Nonstratified	18	0.10 ± 0.05	NT	0.09 ± 0.04	NT
	Stratified	18	NT	NT	NT	NT
Cloz	Fresh	6	0 ± 0	0 ± 0	0.10 ± 0.06	0.57 ± 0.07
	Nonstratified	18	0 ± 0	NT	0.42 ± 0.11	NT
	Stratified	18	1.0 ± 0.0 (0.98 ± 0.02)	1.0 ± 0.0	1.0 ± 0.0 (0.91 ± 0.05)	1.0 ± 0.0
Covi	Fresh	6	0.06 ± 0.06	0 ± 0	0 ± 0	0 ± 0
	Nonstratified	18	0.28 ± 0.17	NT	0.05 ± 0.05	NT
	Stratified	18	0.13 ± 0.13 (0 ± 0)	0 ± 0	0.92 ± 0.08 (0 ± 0)	0.04 ± 0.09

Stratified = 5°C for 12 wks before incubation.

greater in seasonal temperature cycles than the other temperature regimes for *Conradina verticillata* ($P < 0.01$) and *Claytonia ozarkensis* ($P < 0.001$, Fig. 1). In contrast, there were no significant differences in germination fractions among the seasonal temperature cycles and constant temperature regimes for seeds of *Astragalus bibullatus* ($P = 0.14$, Fig. 1).

Germination phenologies varied among species in the seasonal temperature cycles (Fig. 1). In the cold sequence, seeds of *Conradina verticillata* germinated rapidly and to high rates at 15/5 °C following cold (5°C) stratification. Similarly, germination was slow and low in the warm sequence until seeds experienced cold stratification (Fig. 1). In contrast, *Claytonia ozarkensis* germinated to 100% after 12 wks at 5 °C in the cold sequence. In the warm sequence, *C. ozarkensis* germinated at cool and cold temperatures after experiencing summer temperatures. There was no clear germination pattern for *Astragalus bibullatus* in either seasonal temperature sequence (Fig. 1). Due to the overall low germination fractions after one move-along cycle, we subjected *A. bibullatus* seeds to another cycle of seasonal temperatures and found that no additional seeds germinated.

EXPERIMENT 3. *Astragalus bibullatus* seeds subjected to heat (125 °C) for 30 min did not germinate at any combination of temperature and light after 6 wks (Fig. 2). Rather, 90% of *A. bibullatus* seeds subjected to heat lost viability whereas only 11% and 13% of seeds lost viability in the control and scarification treatment, respectively. There were no significant interactions between pretreatment, incubation temperature, and light on *A. bibullatus* germination fractions (all *P*-values > 0.15, Fig. 2). Overall, scarified seeds germinated to greater fractions than the controls. In the scarification treatment, germination rates were significantly ($P = 0.02$) greater at 30/15 °C than at 15/5 °C (Fig. 2) while the effects of light ($P = 0.09$) and the light × temperature interaction were not significant ($P = 0.45$).

Discussion. Freshly matured seeds of the three imperiled rock outcrop endemics varied in their response to temperature and light conditions. At maturity, most *Astragalus bibullatus* seeds exhibit physical dormancy and only a small fraction (< 10%) can germinate in light at warm or cool temperatures. Fresh seeds of *Claytonia ozarkensis* are conditionally dormant and can germinate in darkness at cool but not warm fluctuating temperatures. A majority of fresh seeds of *Conradina verticillata* are physiologically dormant and require cold stratification to germinate. Although the type of seed dormancy varies among the three species, the temperature requirements for dormancy break and germination prevent a majority of seeds of each species from germinating at prevailing habitat temperatures following dispersal.

When considered in conjunction with the timing of seed dispersal, seed temperature responses in the move-along experiment can be used to make predictions on the timing of germination in the wild. *Claytonia ozarkensis* seeds are dispersed in spring but cannot germinate during the summer because warm (≥15 °C) temperatures inhibit germination. As temperatures decrease in late-autumn and winter, *C. ozarkensis* seeds are able to germinate at cool temperature (≤ 15 °C) with

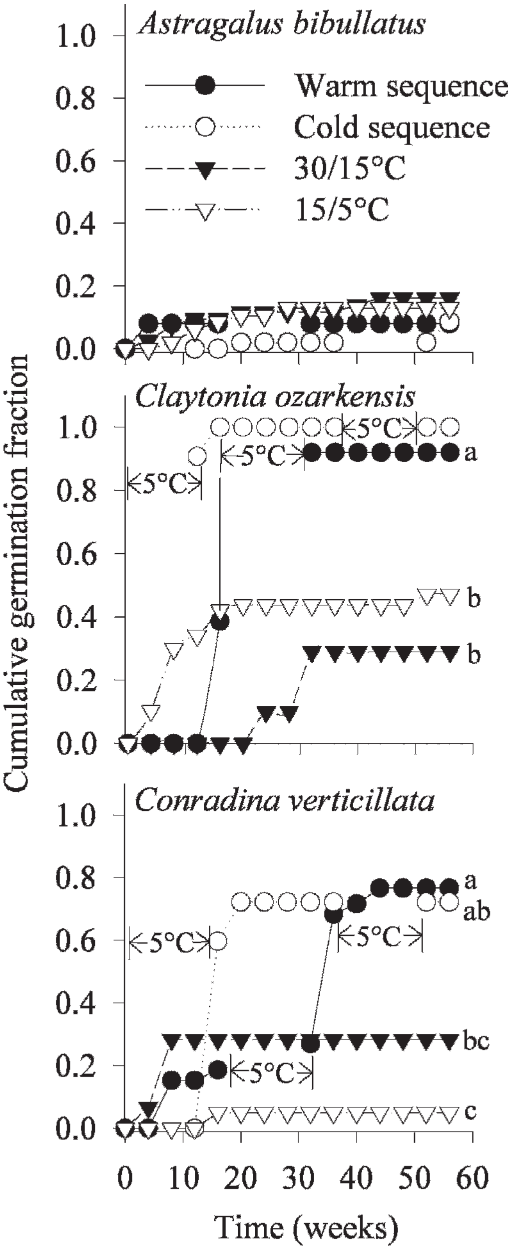

FIG. 1. Cumulative germination fractions for seeds of three imperiled perennials subjected to a move-along experiment. For the warm and cold sequences, lines are broken because germination was not checked while seeds were cold (5 °C) stratified in the dark. Values with different letters indicate significant differences in cumulative germination fractions after 56 wks based on Tukey all pairwise comparison test with Holm adjusted *P*-values. Separate tests were conducted for each species. Error bars are not shown to improve clarity.

maximum germination occurring at low (5 °C) or winter temperatures. Since *C. ozarkensis* germinated to high fractions at 5 °C in the cold sequence of the move-along experiment, seeds do not need to first experience warm moist storage to germinate at low temperatures.

Seeds of *Conradina verticillata* are dispersed in early summer and in our *ex situ* experiments a majority of them germinated after stratification at low temperatures. Unlike in *Claytonia ozarkensis*, though, *C. verticillata* seeds are incapable of germinating at low (5 °C) temperatures. After physiological dormancy is broken by low temperatures, *C. verticillata* seeds germinate rapidly in light at cool fluctuating temperatures that are characteristic of early spring in this species' natural habitat. However, approximately 44% of viable *C. verticillata* seeds are conditionally dormant and can germinate at warm but not cool temperatures immediately following dispersal. Differences in seed dormancy levels among a cohort have been observed in seeds of other temperate members of the Lamiaceae (Albrecht and McCarthy 2006), and could be a strategy to spread germination risks across generations in periodically disturbed habitats (i.e., bet hedging, Venable 1985). In *C. verticillata*'s natural habitat, seedlings that emerge in summer would be prone to drought and competition from surrounding vegetation while seedlings that emerge in early spring may be subjected to periodic inundation (USFWS 1996). Thus, spreading germination across different seasons might be a strategy to increase the probability of successful seedling establishment.

In the only other germination study with *Conradina verticillata* that we are aware of, Roulston (1994) reported that a majority of seeds were nondormant at maturity and germinated at room temperatures in light within 21 d after sowing. However, germination rates were so low that differences between nonstratified and cold stratified seeds could not be detected. In our study, a greater fraction of viable seeds germinated after, rather than before, cold stratification. Also, we observed greater levels of seed viability (26% across all treatments) in our experiment compared to what Roulston (1994) reported (7.5%), indicating that viable seed production varies widely among years and populations in *C. verticillata*.

Differences in the response of *Claytonia ozarkensis* and *Conradina verticillata* seeds to light are consistent with the respective habitat

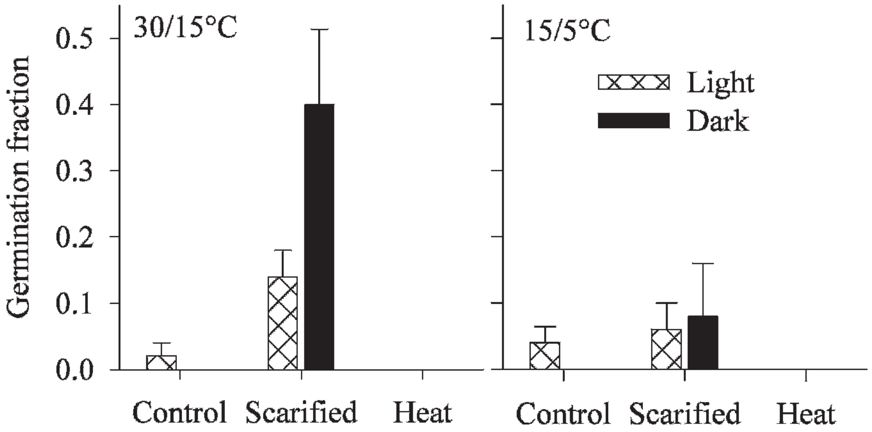

FIG. 2. Germination fractions (mean ± SE) of *Astragalus bibullatus* seeds following heat and scarification treatments. Seeds were subsequently incubated in light and darkness at 30/15 °C and 15/5 °C. No seeds germinated in the heat treatment.

of each species. Seeds of *C. ozarkensis* are dispersed into cracks or fissures of shaded rockfaces (M.A. Albrecht, personal observation), where they probably experience near constant darkness until low temperatures cue germination. The fact that *C. ozarkensis* seeds germinate readily in darkness suggest that few seeds probably persist in the rockface beyond one germination season, resulting in a transient seed bank (*sensu* Walck et al. 2005). Other rockface and cliff endemics are also reported to germinate at greater rates in dark than light (Navarro and Guitian 2003), although it remains unclear whether this pattern is consistent among cliff habitat specialists (Baskin and Baskin 2001). In contrast, *C. verticillata* seeds, which are dispersed primarily by gravity and secondarily by water along floodplains of major streams, germinated to greater fractions in light than in darkness at simulated spring temperatures. For species of temperate habitats, a cold stratification and light requirement for seed germination confines regeneration to gap microenvironments in early spring (Grime et al. 1981, Fenner and Thompson 2005). Indeed, it is hypothesized that *C. verticillata* depends upon periodic flooding, especially during winter months, to maintain an open habitat and create new microenvironments for colonization (USFWS 1996). Furthermore, compared to large-seeded species, small-seeded species, like *C. verticillata*, are more likely to: (1) posses a light requirement for germination (Milberg et al. 2000, Jankowska-Blaszczuk and Daws 2007), and (2) to form persistent soil seed banks (Thompson et al. 1993). Thus, if the appropriate microenvironmental conditions are not met during the narrow spring window when temperatures are suitable for germination, we hypothesize that some *C. verticillata* seeds can carry over to the next germination season in a persistent seed bank.

There was no obvious temperature control over germination timing in *Astragalus bibullatus* seeds. The overall low germination of *A. bibullatus* seeds in simulated natural temperatures were not caused by low seed viability but rather strong physical dormancy that rendered seed coats impermeable to water, consistent with the hypothesis that *Astragalus bibullatus* seeds probably persist in the soil for many years (Morris et al. 2002). In nature, gradual breakdown of the hard seed coat, probably due to temperature and moisture fluctuations, appears to spread germination of *A. bibullatus* seed over many years, a pattern also reported in other annual and perennial legumes endemic to limestone cedar glades (Baskin and Baskin 1989), and other hard seeded species (Baskin and Baskin 2001, Van Assche and Vandelook 2006). Although we could not discern a consistent germination phenology pattern in the laboratory, in nature temperatures may still regulate germination timing of seeds with hard coats (Van Assche et al. 2003, Jayasuriya et al. 2008). For example, we have observed new *A. bibullatus* seedlings in spring in natural populations but only rarely in other seasons suggesting some regulation by seasonal temperatures and (or) germination cues. Other factors known to break physical dormancy, such as animal ingestion (Baskin and

Baskin 2001), seem unlikely since there are no obvious dispersal vectors of *A. bibullatus* seeds (Baskin and Baskin 2005).

Surprisingly, dry heat was ineffective in breaking hard coat seed dormancy in *Astragalus bibullatus* and reduced viability relative to scarified and nonscarified seeds. This contrasts with previous studies that found that dry heat broke dormancy in species that specialize on calcareous grasslands (Bossuyt and Honnay 2008) and in other hard-seeded (physically dormant) perennial herbs where heat has been shown to be just as effective as fire and scarification in breaking physical dormancy (Baskin and Baskin 1997, Baker et al. 2005). Interestingly, the heat shock treatment used in our study was similar to that used by Baskin and Baskin (1998) for seeds of the endangered limestone cedar glade endemic *Dalea foliosa,* which germinated to 48–63% after heating for 20–60 minutes at 100 °C. In our study, no *A. bibullatus* seeds germinated following heat shock, and maximum germination occurred after scarification and then incubation in dark at warm temperatures. However, given that germination fractions in this study and in a previous study that included seed from two other populations (Albrecht and McCue 2010) never exceeded 60%, these findings suggest that some *A. bibullatus* seeds may also have embryo dormancy and that other treatment combinations deserve further investigation.

CONSERVATION IMPLICATIONS. Our *ex situ* germination experiments suggest that the relative importance of processes limiting seedling recruitment in the wild varies among these imperiled species. Although overall viability is low, the few known populations of *Claytonia ozarkensis* produce copious amounts of seeds that germinate readily at low temperatures, suggesting that populations are not limited by a narrow germination niche. However, population recovery following disturbance may be limited by lack of a persistent seed bank. Thus, conservation efforts should focus on the protection of the few known *C. ozarkensis* populations. The *ex situ* germination protocols developed here provide a safety net in case wild populations decline and require augmentation, or if reintroduction is needed to restore this species to its historical range.

On the other hand, natural populations of *Conradina verticillata* are maintained primarily by clonal reproduction and seedling recruitment is rarely observed (USFWS 1996). Our results using seeds from a large population of *C. verticillata* suggest that seed regeneration is constrained primarily by low seed viability, but secondarily by a relatively narrow germination niche. The strict light requirement to cue germination in early spring implies that alteration of disturbance regimes (e.g., flooding) that maintain open microenvironments and prevent woody encroachment could limit seedling recruitment in *C. verticillata* populations. While our work addresses potential factors limiting germination, additional work is required to understand the intrinsic and extrinsic factors causing low seed viability (USFWS 1996). Due to low seed viability, *ex situ* collections of *C. verticillata* will need to include both living collections derived from cuttings and seeds to ensure appropriate levels of genetic diversity are maintained.

Reintroduction is a key component of conservation and recovery efforts with *Astragalus bibullatus* (USFWS 2009). Lack of seedling recruitment contributed to the poor success rates in previous reintroduction attempts; seedling transplants matured and produced seed but few second generation seedlings emerged and survived, resulting in a negative population growth rate (Albrecht and McCue 2010). Our data suggest that physical dormancy is a major limitation to germination in *A. bibullatus* and many years may be needed before dormancy is lost and seedlings emerge from the soil seed bank. Interestingly, at a limestone cedar glade where *A. bibullatus* was previously not known to occur, a large and vigorous population of *A. bibullatus* appeared following mechanical thinning of woody vegetation (USFWS 2009). Presumably, recruitment from a dormant soil seed bank contributed to the emergence of this population, suggesting that microenvironmental changes caused by mechanical thinning stimulated germination (Morris et al. 2002, USFWS 2009). Since heat was ineffective at breaking physical dormancy in our study and we did not observe a light requirement for germination, it remains uncertain what factors might enhance recruitment from the soil seed bank. Given this species' high level of endangerment, field studies investigating the abiotic and biotic factors governing seedling recruitment are therefore urgently needed to guide conservation and management efforts of wild and reintroduced *A. bibullatus* populations.

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