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Author(s): Samara I. Hamzé and Claudia L. Jolls

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## Germination Ecology of a Federally Threatened Endemic Thistle, *Cirsium pitcheri*, of the Great Lakes

SAMARA I. HAMZÉ<sup>1</sup> AND CLAUDIA L. JOLLS<sup>2</sup>

*Department of Biology, East Carolina University, Greenville, North Carolina 27858 and  
The University of Michigan Biological Station, Pellston 49769*

**ABSTRACT.**—Pitcher's thistle (*Cirsium pitcheri*) is a federally listed monocarpic plant species endemic to the shoreline dunes of Lakes Huron, Michigan and Superior. Individual plants may require 4–8 y to mature, after which they flower and die. This life history and the lack of vegetative modes of reproduction make an understanding of seed and seedling ecology critical for preservation and restoration of Pitcher's thistle. We report conditions necessary to overcome seed dormancy and the effects of light, seed mass and depth of burial (0, 2, 4 or 8 cm) on seed and seedling success from laboratory experiments in controlled growth environments. Seeds of Pitcher's thistle are dormant when dispersed, but a combination of low temperatures and afterripening can break dormancy. Germination over 30% was obtained with at least 24 wk of low temperature moist stratification; 25% germination occurred after 6 mo of storage at room temperatures. Light suppressed germination of nondormant seeds. Although burial is required for germination, only 8% of seedlings emerged from a depth of 8 cm, associated with longer time to emergence. Achenes of this taxon lack endosperm; seedlings probably lack the reserves to overcome excessive burial. Logistic regression was used to compare the relative effects of the presence or absence of light, burial depth, seed mass and differences between populations from Michigan's upper and lower peninsulas. All three variables were significant predictors of germination and emergence when tested alone. Seeds from the upper peninsula were more likely to germinate, provided they were buried, although their probability of emergence decreased at greater burial depths. Although seeds from the upper peninsula were significantly heavier on average than those from lower peninsula populations, seed source was an even better predictor of seed and seedling success than seed mass. This suggests genetic differences among populations even within this geographically restricted endemic. Seeds of Pitcher's thistle can remain viable 1–2 y in the laboratory, suggesting this species can maintain a seed bank, although it is ephemeral. Seeds and seedlings of *Cirsium pitcheri* successfully exploit the dynamic nature of their dune habitats. Our results suggest that conservation efforts must consider seed storage conditions, genetic source of seeds and seed size, as well as maintenance of natural sand erosion and accretion regimes for preservation and restoration of this taxon.

### INTRODUCTION

Pitcher's thistle (*Cirsium pitcheri* (Eaton) T. & G., Asteraceae) is a rare plant endemic to the shoreline dunes of the western Great Lakes. Its range extends from southern Lake Michigan to the northern shore of Lake Superior (Pavlovic *et al.*, 2000). Most *C. pitcheri* populations are found on the eastern shore of Lake Michigan, where longshore currents and climatic patterns are favorable for maintaining the appropriate open sand habitat (McEachern, 1992).

Since the turn of the century at least six known populations have been extirpated from the southern extreme of the range of *Cirsium pitcheri* (McEachern, 1992) and two from the eastern shore of Lake Huron (Keddy, 1987). Recently, this globally rare species (G3)

<sup>1</sup> Present address: Archbold Biological Station, P.O. Box 2057, Lake Placid, Florida 33862

<sup>2</sup> Corresponding author: e-mail: jollsc@mail.ecu.edu

gained federal protection as a threatened taxon because its habitat is threatened (Harrison, 1988; Pavlovic *et al.*, 2000). The successful protection of Pitcher's thistle depends on greater knowledge of its biology and habitat.

*Cirsium pitcheri* is a monocarpic perennial that persists in rosette form for 4–8 y (Loveless, 1984) before flowering once and then dying. Pitcher's thistle only reproduces sexually. Thus, this species' monocarpic life history, plus the absence of vegetative reproduction, make ecesis critical for its persistence. Seedling emergence begins in late May (Loveless, 1984) and continues through July (pers. obs.). In the first season true leaves are entire and densely tomentose. In subsequent years rosette leaves are pinnatifid and tomentose. Reproductive plants are easily identified by the flowering stems that begin to bolt in May and reach an average height of  $86.5 \pm 4.87$  cm (mean  $\pm$  SE, pers. obs.). On average there are 1–35 heads per plant of only disk florets (Loveless, 1984) and  $85.1 \pm 3.09$  florets per head ( $n = 35$ , pers. obs.) which produce achenes (hereafter referred to as "seeds"). Flowering is determinant and flowers range from creamy white to light pink. Flowers can self-pollinate but cross-pollination by a variety of bees and moths results in higher seed set (Loveless, 1984; McEachern, 1992). Pitcher's thistle seeds are the largest of any North American thistle (Gleason, 1952).

The dune habitat of *Cirsium pitcheri* imposes several important stresses on plants: (1) low water availability, (2) heat stress and (3) burial (Barbour *et al.*, 1985; Maun, 1994). Like other Great Lakes dune natives (Sykes and Wilson, 1990; Zhang and Maun, 1990; Maun, 1994) and congeners (van der Valk, 1974; Zedler *et al.*, 1983), *Cirsium pitcheri* appears to be well-adapted to its sandy arid environment. Adults of the species have a well-developed taproot that provides escape from heat, access to moisture and anchors the plant in its shifting sandy habitat.

Although some aspects of Pitcher's thistle life history have been studied (Keddy and Keddy, 1984; McEachern, 1992; D'Ulisse and Maun, 1996; Stanforth *et al.*, 1997), knowledge of its reproductive biology is limited. Since *Cirsium pitcheri* does not reproduce vegetatively, species persistence depends on successful seed production, seed germination and seedling establishment. The success of restoration efforts such as those begun at Illinois Beach State Park (Bowles and McBride, 1996) is dependent upon our knowledge of seed and seedling ecology of this taxon.

Pitcher's thistle seeds are dormant at the time of seed dispersal in the fall (Pavlovic *et al.*, 2000). Seeds of other taxa generally require a combination of afterripening, low temperature moist stratification, abrasion or chemical leaching to overcome innate dormancy (Fenner, 1985). Once dormancy is broken appropriate conditions for germination are required. Light inhibits germination in some dune species (Maun and Lapierre, 1986). Several studies have indicated that, within a species, seeds of higher mass also have increased probabilities of germinating and emerging from depth (Maun and Lapierre, 1986; Weller, 1989; Zammit and Zedler, 1990; Maun, 1994; Prinzie and Chmielewski, 1994; Andersson, 1996).

Some other dune taxa reproduce sexually when sand movement is high, suggesting seeds may be adapted to burial (Barbour *et al.*, 1985). Maun and Lapierre (1986) found that the four Great Lakes dune species they studied all required burial for maximum germination; D'Ulisse (1995) found that *Cirsium pitcheri* seedlings can tolerate repeated burial of 50% maximum leaf length. In addition, other dune species (*e.g.*, *Abronia maritima*) show greater root growth in areas of higher sand accumulation vs. those with lower sand accumulation (Johnson, 1978). Pitcher's thistle must possess mechanisms for tolerating the moving sands of the dynamic dune environment. For seeds in the arid dune environment burial may

confer advantages such as providing seeds protection from predation and reducing chances of desiccation (Maun, 1994).

Our intent was to study the seed ecology of *Cirsium pitcheri* and to aid reintroduction efforts (Bowles and McBride, 1996). We studied two aspects of the life history of *C. pitcheri*, (1) conditions necessary for breaking seed dormancy and (2) the effects of light, seed mass and depth of burial on seed germination and seedling success. We also document variation in seed germination and seedling emergence among populations and suggest how the seed ecology of Pitcher's thistle helps maintain populations in the dynamic dune habitat.

#### METHODS

*Sites.*—Pitcher's thistle seed heads were collected by permit during the first 2 wk of Aug. 1996 from each of three sites: Sturgeon Bay (NE ¼, Sec. 5, T 38 N, R 5 W) and Waughoshance Point (SE ¼, Sec., 19, T 39 N, R 5 W) in Wilderness State Park in Michigan's lower peninsula (LP population) and Pictured Rocks National Lakeshore (Sec. 7, T 49 N, R 14 W) in the upper peninsula (UP population); the two LP populations were later combined because they did not differ significantly in size. Heads were harvested from the primary or secondary stem and stored individually in manila envelopes at room temperature until used. Because of the threatened status of this species, collections were limited to only one head per plant from populations in excess of 200 plants. For both germination studies small and obviously inviable seeds were removed.

*Seed dormancy.*—In a pilot study we tested the effects of low temperature, moist stratification and age on seed dormancy. Seeds from the lower peninsula were germinated after two pregermination treatments: stratified (moist chilled in the dark at 2 C using sand-filled petri dishes) or unstratified (stored at room temperature in manila envelopes). We began stratification 2 wk after head collection. Thus, when 12 wk seeds were tested for germination, stratified LP seed had 10 wk of moist chilling. Every 2 wk through week 14, then every 4 wk from week 20–32 after harvest, 20–40 seeds from each storage treatment were selected without bias to test for germination. This method allowed for control of seed age as well as duration of stratification. Seeds were germinated by placing them on moist autoclaved sand in 9 cm petri dishes in a Conviron E-15® (Pembina, ND) growth chamber at 30/15 C, 15/9 h light/dark at 95  $\mu\text{moles m}^{-2} \text{s}^{-1}$  photosynthetic flux density; water was added as needed. Dishes were checked daily for seed germination, defined as the penetration of seed coat by the radicle. Viability of ungerminated seeds was tested either by cutting the fruit coat of ungerminated seed and staining with tetrazolium (Colbry *et al.*, 1961) or by squeezing the achene with forceps to indicate presence of a firm embryo (Baskin and Baskin, 1998). A random selection of 52 ungerminated seeds confirmed squeezing the achene to be 100% accurate in determining viability; all 52 firm achenes contained viable embryos, verified using tetrazolium stain. Virtually all germination occurred within the first 2 wk; the experiment was terminated after 4 wk.

*Seed mass.*—Fertilized ovules (filled seeds, recognized by the dark fruit coat surrounding a plump seed) were separated from unfilled seeds and unfertilized ovules. Seed heads were dissected under 10X magnification. We selected filled seeds, 192 from each of the LP population sites (384 total) and 469 from the UP population, without bias, from a total of 149 heads. Seeds then were weighed individually to the nearest 0.001 mg on a Cahn® Model 31 microbalance (Cerritos, CA).

*Seed burial.*—Thirty-two wk after harvest 320 seeds that had been moist stratified for 14 wk were randomly assigned to one of four treatments: 0 cm (no burial), 2 cm, 4 cm or 8 cm burial depths. This stratification period and these depths of burial were selected based on previous studies (Baskin and Baskin, 1998). Seeds used in this experiment were selected

TABLE 1.—Maximum percent germination under controlled conditions (30/15 C, 15/9 h light/dark) for *Cirsium pitcheri* seeds collected in 1997 from Michigan's lower peninsula (LP). Numbers in parentheses indicate the number of seeds used. Duration of stratification for seeds was 2 wk less than seed age. All seeds less than 24 wk after harvest had less than 10% germination

Age (weeks)	Unstratified	Stratified
24	1.3 (80)	35.0 (60)
28	0.0 (80)	22.5 (40)
32	0.0 (80)	27.5 (40)

without bias from seeds weighing more than the population mean: over 9.05 mg for the 160 LP seeds and over 12.41 mg for the 160 UP seeds. Seeds heavier than the mean were selected to maximize seed germination, thus permitting us to determine the effects of light, mass, depth of burial and population on germination and emergence. Seeds were planted at the appropriate depth in 2 cm × 2 cm × 20 cm cells (Root-Trainer®, Hummert Intl., Earth City, MO) filled with autoclaved Lake Michigan dune sand. The cells were placed in a Conviron® E-15 growth chamber, again at 30/15 C, 15/9 h light/dark, 95  $\mu\text{moles m}^{-2} \text{s}^{-1}$  photosynthetic flux density. Cells were watered daily to maintain field capacity and rotated twice weekly to minimize position effects.

We checked daily for cotyledon emergence for 35 d. If no seedling emerged, seeds were retrieved to determine whether germination had occurred, but the seedling failed to emerge. As in the seed dormancy experiments, presence of a viable embryo was confirmed either with tetrazolium stain or by squeezing the achene with a pair of forceps. In cases where a seed was not found we assumed it had not germinated. This occurred for only 28 of the 320 seeds used.

Student's *t*-tests, analysis of variance on rank transformed data or their nonparametric equivalents were used to compare means (*e.g.*, mass or number of days to emergence between populations and among depths). Chi-square contingency tests were used to determine if frequencies of germination and emergence differed between LP and UP populations and among depths. Both germination and emergence are binary events (either the seed did or did not germinate or emerge). Thus, we used logistic regression tests to compare the effects of light (surface = 0, buried = 1), seed mass, depth of burial (2 cm, 4 cm, 8 cm) and population (LP = 0, UP = 1) on the probabilities of seed germination and seedling emergence. This statistical approach can be used to identify predictors of germination (Jolls and Chenier, 1989; Andersson, 1996). All statistical analyses were performed using SPSS 7.5.1 (SPSS, 1997).

## RESULTS

*Seed dormancy.*—Data from the petri dish experiments compared effects of storage temperature and age on germination (Table 1). Germination was greatest 24 wk after harvest and did not exceed 10% for seeds that had afterripened no more than 20 wk. Moist stratification increased germinability of LP seeds to 35% after 22 wk of stratification. Unstratified seeds virtually never germinated in the growth chamber.

*Seed mass.*—The UP population had significantly heavier seeds on average than either LP site ( $F_{2, 850} = 195.485$ ,  $P \leq 0.001$ ). Mean mass of seeds from the two LP sites did not differ statistically (mean  $\pm$  SE =  $8.86 \pm 0.14$  mg vs.  $9.24 \pm 0.14$  mg, SB vs. WP, respectively;  $t = 1.917$ ,  $df = 382$ ,  $P = 0.06$ ) and LP seeds were subsequently treated as one population.

TABLE 2.—Percentages of seed germination and seedling emergence for *Cirsium pitcheri* seeds (32 wk after harvest, including 14 wk of moist stratification) from Michigan's lower (LP) and upper (UP) peninsulas in controlled laboratory conditions (30/15 C, 15/9 h light/dark). Sample size = 40 for each treatment within each population

Depth of planting (cm)	Total germination (%) Site		Total emergence (%) Site	
	LP	UP	LP	UP
0	0.0	25.0	0.0	22.5
2	45.0	72.5	45.0	72.5
4	10.0	72.5	10.0	67.5
8	32.5	82.5	7.5	35.0

However, seeds from the upper peninsula still were significantly heavier than those from the two lower peninsula populations combined (mean  $\pm$  SE = 12.41  $\pm$  0.13 vs. 9.05  $\pm$  0.10 mg, UP vs. LP, respectively;  $t = 20.421$ ,  $df = 382$ ,  $P \leq 0.001$ ).

**Seed burial.**—Seeds in the burial experiment were 32 wk old after harvest and had been moist stratified in the dark for 14 wk; overall germination was 42.5%. Of the 320 seeds planted in the burial experiment 3 did not germinate because they had decomposed and 28 could not be relocated. All of the remaining 289 seeds that were retrieved were found to contain a firm embryo, determined by squeezing the achene with forceps.

Lack of burial suppressed germination. For both the LP and UP populations germination and emergence of unburied seeds (0 cm) were low (<13%). Overall, 52.5% of seeds that were in the dark (buried) germinated (Table 2).

Mass also affected seed germination. On average, seeds that germinated weighed 13.56  $\pm$  2.33 mg; those that did not germinate weighed 11.97  $\pm$  2.43 mg. This difference was significant ( $t = 5.89$ ,  $df = 318$ ,  $P \leq 0.001$ ). However, the mean masses of germinated seeds for seedlings that did vs. did not emerge were not significantly different (13.59 mg and 13.46 mg, respectively,  $t = 0.26$ ,  $df = 134$ ,  $P = 0.79$ ).

In general, emergence decreased with an increase in burial depth. Unexpectedly, germination of buried seeds differed among depths: 47/80 (59%) at 2 cm, 33/80 (41%) at 4 cm and 46/80 (58%) at 8 cm (Table 2;  $\chi^2 = 6.115$ ,  $df = 2$ ,  $P \leq 0.05$ ). This lack of pattern in the frequency of germination with depth was due to the low number of LP seeds that germinated at 4 cm (4/40), compared to higher germinability at 2 cm (18/40) and 8 cm (13/40).

Frequencies of seedling emergence also differed among the three burial depths (Table 2;  $\chi^2 = 23.446$ ,  $df = 2$ ,  $P \leq 0.001$ ) as did average number of days to emergence (mean  $\pm$  SE = 8.34  $\pm$  0.67 at 2 cm, 11.07  $\pm$  0.62 at 4 cm and 13.88  $\pm$  1.97 cm at 8 cm, Kruskal-Wallis = 25.404,  $df = 2$ ,  $P \leq 0.001$ ). Emergence of seedlings decreased with increasing depth; only 17 of 46 (37.0%) germinated seeds emerged from 8 cm (Table 2).

Mass, germination and emergence differed between LP and UP populations. Seeds from the UP were significantly heavier than LP seeds, with a significantly higher frequency of germination (101/160 (63.1%) vs. 35/160 (21.9%);  $\chi^2 = 55.70$ ,  $df = 1$ ,  $P \leq 0.001$ ). Also, significantly more UP seedlings emerged compared to LP seedlings (79/160 (49.4%) vs. 25/160 (15.6%);  $\chi^2 = 41.538$ ,  $df = 1$ ,  $P \leq 0.001$ ).

Light, mass, depth of burial and population each were used individually as the predictor variable in logistic regression tests. Each was a significant predictor of either germination or emergence when tested alone. The probabilities of germination increased for seeds that

TABLE 3.—Results of two logistic regressions comparing (a) light (0 or 1), seed mass and population (LP or UP) to the likelihood of *Cirsium pitcheri* germination and (b) the influence of seed mass, depth and population on the likelihood of *C. pitcheri* emergence from 2, 4 or 8 cm burial. B = coefficient, Wald = (B/SE)<sup>2</sup>, used to compute the test statistic distributed as a chi-squared, *e* = base of natural log. All other Wald statistics exceeded P ≥ 0.05

Variable	B	SE	Wald	df	P	<i>e</i> <sup>B</sup>
(a) Germination						
Light	2.4654	0.3977	38.4328	1	0.0000	11.769
Mass	-0.0859	0.1005	0.7312	1	0.3925	0.9176
Population	2.5061	0.5192	23.2986	1	0.0000	12.257
Constant	-2.5041	1.1393	4.8308	1	0.0280	
(b) Emergence						
Mass	-0.0142	0.1126	0.0158	1	0.8999	0.9859
Depth	-0.3261	0.0670	23.5938	1	0.0000	0.7218
Population	1.9563	0.5677	11.8739	1	0.0006	7.0729
Constant	0.1491	1.2061	0.0153	1	0.9016	

were buried (*i.e.*, in the dark), for heavier seeds and for UP seeds. However, when the 0 cm depth was excluded depth of burial was not a significant predictor of probability of germination (B = 0.0262 ± 0.0519, Wald = 0.0967, df = 1, P = 0.7559).

When light, mass and population all were used as predictor variables in logistic regression tests for the probability of germination, light and population were significant predictors (Table 3a). Depth of burial (≥2 cm) was not included in the regression since it was not a significant predictor when tested alone. The logistic regression for germination indicated that seeds that were buried (*i.e.*, in the dark) had a greater probability of germinating.

We also were interested in determining the effects of depth of burial on the probability of emergence. When mass, depth and population were used as the predictor variables, depth and population were significant predictors of emergence (Table 3b). Like the probabilities of germination, the probabilities of emergence increased for buried seeds, for heavier seeds and for seeds from the UP. Although depth of burial had no effect on probability of germination, probability of emergence decreased with an increase in burial depth.

We used these logistic regression models to calculate the probabilities of both germination and emergence (Table 4). The values 0 and 1 were used for the variables light (0 = surface, 1 = buried) and population in the logistic regression model: probability of ger-

TABLE 4.—Probabilities of germination and emergence of *Cirsium pitcheri* seedlings from two populations and three burial depths. LP = lower peninsula, UP = upper peninsula. The probability of germination was calculated as  $1/(1 + e^{-Z})$ , where  $Z = -2.5041 + 2.4654(\text{light}) + 2.5061(\text{population})$ . The probability of emergence was calculated as  $1/(1 + e^{-Z})$ , where  $Z = 0.1491 - 0.3261(\text{depth}) + 1.9563(\text{population})$

Popu- lation	Probability of germination Light		Probability of emergence Depth (cm)		
	Yes	No	2	4	8
LP	0.0756	0.4903	0.3768	0.2395	0.0787
UP	0.5001	0.9218	0.8105	0.6902	0.3767

mination =  $1/(1 + e^{-Z})$ , where  $Z = -2.5041 + 2.4654 (\text{light}) + 2.5061 (\text{population})$ . The values 2 cm, 4 cm and 8 cm for the variable depth and 0 = LP, 1 = UP for the variable population were used in the logistic regression model: probability of emergence =  $1/(1 + e^{-Z})$ , where  $Z = 0.1491 - 0.3261 (\text{depth}) + 1.9563 (\text{population})$ . For both populations, buried seeds had lower chances of germinating; probabilities of emergence also decreased with greater burial depth. We found that buried LP seeds (light = 1, population = 0) had nearly the same probability of germinating as surface seeds from the UP (light = 0, population = 1). We also observed 90% probability of germination for buried UP seeds. Seeds from the UP buried at 2 cm had the highest probability of emerging (81.1%). The lowest probability of emergence was for seeds from the LP buried at 8 cm (7.9%). It is interesting to note that the lowest probability of emergence for UP seeds (37.7%) was equal to the highest probability of emergence for LP seeds.

#### DISCUSSION

Like seeds of the congeners, *Cirsium canescens* (Lamp and McCarty, 1981) and *C. arvense* (LaLonde and Roitberg, 1994), Pitcher's thistle seeds are dormant at the time of dispersal in the fall and dormancy is broken by a combination of afterripening and moist chilling. Seeds tested for dormancy and germination had less than 10% germination until 24 wk after dispersal (roughly equivalent to the month of January). After stratification seeds germinated and seedlings emerged within 2 wk. Chen and Maun (1998) found cold temperatures can enhance germination of fruit from *C. pitcheri* collected from garden-reared individuals. In the field delayed germination of seeds allows seedlings to emerge when moisture and temperature are optimal for seedling success and when competition for light is low (Fenner, 1985; Leck *et al.*, 1989).

The relative effects of seed age and length of stratification on breaking dormancy are unknown; these variables were confounded in our experiments. However, a minimum of 14 wk of chilling, 18 wk after harvest, was required for germination in excess of 50%, based on our burial experiment. We observed germinability over 70% for buried UP seeds, yet UP seeds, stratified at 6 wk of age, can fail to germinate to any significant extent in the dark, even when stratified (pers. obs.). One possible explanation may be the age at which stratification is administered; an "artificial early winter" may press seeds into an early enforced or induced (secondary) dormancy (Nikolaeva, 1977; Bewley and Black, 1985). The influence of temperature, particularly low temperatures, on germination merits further study. During stratification (2 C) we observed germination in excess of 50% for afterripened LP seeds (26 wk old, including 18 wk of stratification). *Cirsium pitcheri* may be yet another member of the Asteraceae for which the minimum temperatures for germination decrease during afterripening (Type 2 response of Vegis, 1964), observed in 22 of 32 species of the family tested (Baskin and Baskin, 1988; Buchele *et al.*, 1991; Baskin *et al.*, 1993). Chen and Maun (1999) also reported greater dormancy in large compared to small seeds of *Cirsium pitcheri*; also a small proportion of seeds acquired dormancy at deeper depths in their burial experiment.

Pitcher's thistle seeds required burial for more successful germination and emergence. The logistic regression analyses indicated: (1) the greatest probability of germination was for buried seeds (*i.e.*, seeds in the dark), (2) seeds at 2 cm had the greatest probability of emergence, (3) mass was not a significant predictor of germination or emergence and (4) probabilities of both germination and emergence were greatest for seeds from the upper peninsula. We observed relatively low germination ( $\leq 35\%$ ) in the seed dormancy experiment, even if seeds were stratified; these seeds were exposed to light in a 15/9 photoperiod. Furthermore, in the seed burial experiment, seeds placed on the sand surface either failed



to germinate (LP) or had very low germination (UP, 25%), but over 60% of buried seeds germinated. In studies of other dune species (*Ammophila breviligulata*, *Cakile edentula*, *Cirsium rhizophyllum*, *Corispermum hyssopifolium* and *Elymus canadensis*), seeds of all plants required burial for successful seed germination (Zedler *et al.*, 1983; Maun and Lapierre, 1986). *Cirsium pitcheri* reintroduction experiments at Illinois Beach State Park (Bowles and McBride, 1996) also indicated a burial requirement for successful germination.

Enhanced germination of buried *Cirsium pitcheri* seeds can be related to several environmental cues, however, the most obvious is darkness (Baskin and Baskin, 1998). Reduced germination of seeds at the surface is unlikely to be related to changes in availability of carbon dioxide or oxygen. Soil carbon dioxide is not very important in changing dormancy states (Baskin and Baskin, 1998) and carbon dioxide levels are virtually unchanged within the top 10 cm of the soil (Richter and Markewitz, 1995). If oxygen levels decreased with increasing soil depth in our burial experiment we would have expected reduced germination at depth, rather than our observed enhanced germination. Seeds of Pitcher's thistle at the soil surface may have suffered moisture stress, known to reduce germination of *Cirsium arvense* (Wilson, 1979). Germination of *Senecio jacobaea* was inhibited in full light on the sand surface where moisture levels were reduced (van der Meijden and van der Waals-Kooi, 1979). However, we watered daily to maintain the substrate at field capacity.

Lower germination of seeds in the dormancy experiment with 15/9 h photoperiod and the enhanced germination of buried seeds suggest that light may inhibit germination of *Cirsium pitcheri*, more so than other environmental factors. However, germination, albeit reduced, can occur in light. Chen and Maun (1998) reported 100% germination of *C. pitcheri* achenes nicked at the cotyledon end, even in the light; these seeds were at least 7-month old and from garden-reared adults. They concluded that light did not significantly influence germination of nicked seeds, rather the fruit coat poses a physical constraint to emergence of the embryo radicle. Similarly, we observed a maximum of 35% germination of stratified seeds exposed to light. Light may interact with other environmental factors such as scarification, temperature and the age of the achene to influence germination success.

Lack of burial may further limit ecesis by increasing seedling mortality of the few seeds that do germinate at the surface. Similar to our results, when seeds of other dune taxa germinated at the sand surface, seedling radicles could not penetrate the sand (Zedler *et al.*, 1983; Maun and Lapierre, 1986). Burial provides seeds escape from predation and desiccation and places them in a favorable microenvironment where moisture and temperatures are more constant (Maun, 1994).

The advantages of burial were balanced by the disadvantage of excessive burial. Greater depth of burial had a negative effect on seedling success of *Cirsium pitcheri*, as found for other dune taxa (Maun and Lapierre, 1986). Fewer seedlings emerged and took longer to emerge from 8 cm than from 2 cm of burial. While germination of buried seeds was high, emergence of seedlings decreased with increasing depth, suggesting that seeds lack the resources to overcome excessive burial. Zedler *et al.* (1983), Maun and Lapierre (1986) and Chen and Maun (1999) reported similar results for their burial experiments. Burial at too great a depth inhibited seedling emergence; however, the maximum depth from which seedlings could emerge differed among species. Burial depths below 8 cm significantly depress both germination and emergence (this study and Chen and Maun, 1999).

Our burial experiment suggested that *Cirsium pitcheri* may maintain a seed bank, although their existence in xeric dune habitats has been disputed (Planisek and Pippen, 1984; Leck *et al.*, 1989). Zhang and Maun (1994) suggested that the seven dune species they studied maintained seed banks; in their view seeds remain dormant until a disturbance to

the soil brings the seeds closer to the sand surface. In the case of Pitcher's thistle, if there is a seed bank, it is short-lived; dormant seeds form a transient seed bank from dispersal in the fall through the unfavorable conditions of winter until germination in the spring. This Type 2 response (Vegis, 1964) of afterripening and germination relative to temperature is an adaptation to a cold unfavorable season (Baskin *et al.*, 1993). Ungerminated seeds were still viable after our burial experiment, suggesting that these seeds could germinate. Bowles and McBride (1996) also found that *C. pitcheri* can germinate 2 y after seed dispersal. Further investigation is required to determine if Pitcher's thistle maintains a persistent seed bank.

Seed mass and differences among populations both influenced seed and seedling success of *Cirsium pitcheri*. On average, seeds that germinated were heavier than seeds that did not germinate. Seeds from Michigan's upper peninsula were 25% heavier, on average, than those from the lower peninsula and exhibited higher germinability in the burial experiment. Other studies have demonstrated the positive influence of seed mass on seedling success (Harper and Obeid, 1967; Maun and Lapierre, 1986; Weller, 1989; Maun, 1994; Prinzie and Chmielewski, 1994). However, the mean mass of germinated *C. pitcheri* seeds from which seedlings emerged did not differ from that from which seedlings failed to emerge. Similarly, Chen and Maun (1999) showed that germination and emergence of *Cirsium pitcheri* in the greenhouse did not differ among three seed size classes. This suggests factors other than seed mass influence seed and seedling success. In fact, the logistic regression analyses indicated that seed source (LP vs. UP populations) was a better predictor of seed and seedling success than was seed mass. Chen and Maun (1998) also found that seeds of *C. pitcheri* garden-reared from Manitoulin Island collections from Lake Huron did not exhibit different mean germination, despite differences in average mass (6.18 vs. 8.74 mg/seed). We note these achene sizes are significantly smaller than those we work with from Lakes Michigan and Superior. Weller (1985) suggested variation in seed mass of *Lithospermum carolinense* is genetically based. In our study seeds of Pitcher's thistle from the upper peninsula of Michigan were more successful than those from the Straits of Mackinac region, even when controlling for differences in seed mass.

*Cirsium pitcheri* may possess adaptive genetic variation among populations, despite limited levels of polymorphism, as found for other endemic taxa (Karron *et al.*, 1988). Bowles and McBride (1996) suggest genetic differences between parental seed sources may explain differences in success of *C. pitcheri* seedlings from Indiana and Wisconsin. This also may be the case for *C. pitcheri* seeds from Michigan's upper and lower peninsulas. In allozyme tests Loveless (1984) noted that *C. pitcheri* populations from the Straits of Mackinac region (comparable to our LP seeds) had distinctive allele frequencies compared to other populations from throughout the range. Matings between nearby populations of other geographically restricted taxa often result in a decrease in fitness, possibly due to differences in phenology, pollinator movements and sensitivity to inbreeding (Morán-Palma and Snow, 1997; Byers, 1998). Even within a rare endemic like Pitcher's thistle, plant populations may be regionally adapted to local regimes of abiotic and biotic selective pressures. Whether the observed differences between LP and UP seeds are a genetic or phenotypic response to environmental differences merits further study. Managers must consider seed source when selecting seeds for recovery efforts.

To date, successful reintroduction of the federally threatened *Cirsium pitcheri* has largely consisted of yearly planting of seedlings (Bowles *et al.*, 1993; Bowles and McBride, 1996). Establishment of new populations from introduced seeds has had low success due to low emergence of seedlings in the field ( $\approx 2\%$ , Loveless, 1984; Ziemer, 1991; Bowles and McBride, 1996). Our study documents mechanisms for seed germination and seedling es-

tablishment that can be used to maximize seedling success. Germination success can be maximized by selection of large seeds. The effect of seed mass on germination or emergence was insignificant compared to seed age, storage or germination conditions or differences among populations. However, our experiments in the laboratory did achieve >50% germination by selecting seeds larger than the population mean (>9.41 mg). This is in contrast to other reports of less than 5% germination in the field and 22% in the greenhouse (Loveless, 1984; Ziemer, 1991; Bowles and McBride, 1996). Achenes under a critical mass will not germinate, presumably due to absence of a viable embryo.

After selection of seeds, afterripening and stratification must occur. Germinability was greatest for seeds that were at least 24 wk old, cold stratified and buried. If stratification occurs, naturally or artificially, germination can increase significantly ( $\approx 72\%$  recorded in 1981 by Loveless (1984) for seeds buried in the field, 30–40% for seeds overwintering in soil in the greenhouse, M. Bowles, pers. comm.). Stratification of *Cirsium pitcheri* can be started soon after harvest, in early September, and seeds germinated 6 mo later. However, we found greater consistency between geographically disparate populations (LP and UP) when seeds afterripened for 4 mo and stratification was started in early December, as in our burial experiment. This latter treatment is more consistent with field conditions. Germination can then be initiated in early spring (April) by burying seeds at 2 cm. Given this pregermination treatment, the period of afterripening is roughly equivalent to the duration of the winter months in the Great Lakes dunes. Thus, it is possible that seeds can successfully age and stratify in the field. However, to maximize emergence in the spring, site selection must provide burial of seeds, yet sand accretion cannot exceed 8 cm for seedlings to emerge; optimal accumulation would be 2 cm.

The dormancy and burial requirements that we observed for *Cirsium pitcheri* suggest that this species exploits the dynamic nature of its native habitat to maximize seedling success. Seedling stress is avoided by delaying germination until the spring. This insures that seeds germinate when temperature and moisture are optimal for seedling success (Maun, 1994; Chen and Maun, 1998). The burial requirement for seed germination also protects seeds from desiccation and predation and places them in a microenvironment where temperature and moisture are relatively constant (Westelaken and Maun, 1985; Lee, 1993).

The long term protection and propagation of Pitcher's thistle depend on the protection of natural sand erosion and accretion regimes, both necessary for maintaining open habitat. Sand movement allows for seed burial which can confer the appropriate environment for seed germination and seedling emergence. Dune stabilization and alteration of longshore currents and sand deposition (*e.g.*, jetty or marina construction) threaten conditions necessary for seedling establishment critical to the maintenance of this rare thistle.

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