

# Initial Transplant Size and Microsite Influence Transplant Survivorship and Growth of a Threatened Dune Thistle

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## ABSTRACT

Identifying optimal transplant size and microsite requirements are critical challenges facing restoration practitioners, and refining this information may lead to more cost effective decisions. In this study, we evaluate the effects of transplant size and microsite on restoration success of the federal threatened *Cirsium pitcheri* (Pitcher's thistle), a monocarpic perennial restricted to shoreline sand dunes of the Great Lakes in the United States, using a ten-year dataset. Using general and generalized mixed linear models, we determined how microsite variables influenced first-year-transplant survival and subsequent growth. Our data show a higher probability of first-year survival associated with larger transplants. We also found greater plant growth at higher elevations while plants on steeper slopes are smaller the year after they are transplanted. These results have implications for restoration success, which may be maximized by regulating transplant size and selecting habitat.

**Keywords:** *Cirsium pitcheri*, dune topography, Great Lakes, plant reintroductions, restoration

## Restoration Recap

- Finding suitable sites for restorations has always been preferred yet a challenging concept.
- We evaluated the impact that transplant size and habitat characteristics had on transplant success for the federally threatened *Cirsium pitcheri*.
- In the context of a single reintroduction, the best option for transplant success is ensuring the first-year survival of transplants by increasing transplant size. However, it is important to consider whether there is a flowering threshold for the target species as this may impact future reproduction capabilities.
- Plant growth in the year following transplantation depends primarily on the characteristics of the habitat. In this study, transplants grew larger at higher elevations and on flat surfaces. These characteristics likely reduce sand burial and sand erosion.
- Utilizing existing data on restorations can improve the science of restoration; therefore, it is important to analyze results of previous restoration experiments in the context of differences in habitat.

Restorations are often used as the last resort to prevent extinction and maintain species genetic diversity (Maunder 1992, Bowles and Whelan 1994, Falk et al. 1996) when habitat protections and other management strategies fail (White 1996, Maunder 1992, Bowles and Whelan 1994, Falk et al. 1996). However, when reintroducing a species,

practitioners face two primary challenges: identifying optimal habitat (Maschinski et al. 2004, Wendelberger and Maschinski 2009, Albrecht and McCue 2010, Bowles et al. 2015) and choosing appropriate propagation and planting methods (Maschinski et al. 2012). Identifying planting methods that produce greater survivorship is critical. There are two methods typically used in plant restorations: seed broadcast and out-planting of greenhouse-grown transplants. Transplants tend to have a higher success rate than plants started with seeds, especially in high-stress systems such as dune ecosystems (Kaye 2004, Godefroid et al. 2011, Albrecht and Maschinski 2012, Dollard and Carrington 2013).

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Dune ecosystems are subject to environmental stresses from sand burial, drought, high temperature, nutrient limitation, and erosion (Hesp 1991, Maun 1998). This leads to vegetation zonation and succession (Feagin et al. 2005, Forey et al. 2008). As a result, identifying appropriate microsites that provide optimum habitat conditions may be critical in dune systems in order to successfully establish a species (Marteinsdóttir et al. 2013). Topography can influence microsite variables that in turn affect sunlight, water availability, and temperature, thereby, determining whether plants can colonize and persist (Maschinski et al. 2012). Knowing the precise microsite requirements of a species can allow researchers to direct restoration efforts in a way that is more effective both in terms of time and cost (Pavlik 1996). As a result, experimental planting across environmental gradients may help reveal microsite requirements allowing for practitioners to optimize the establishment, reproduction, and survival of individuals (White 1996).

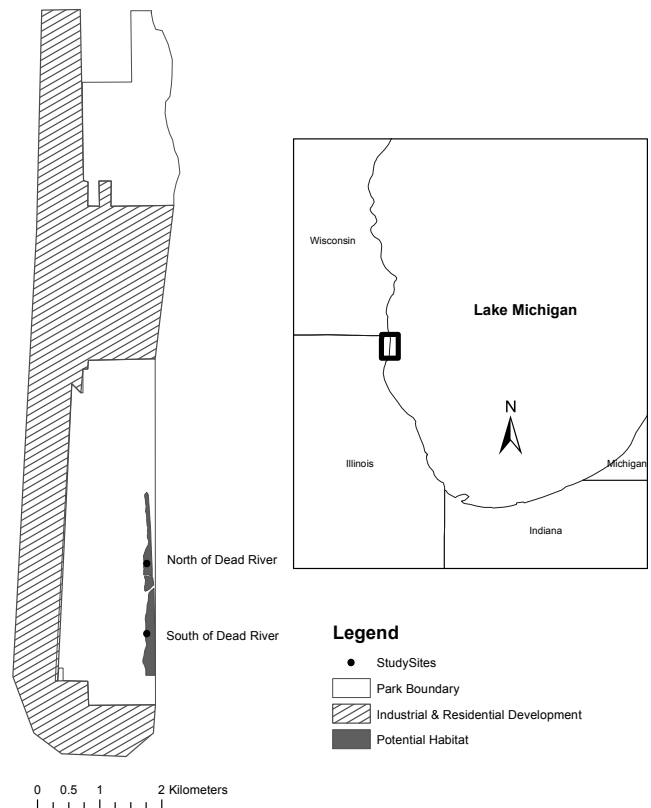
The Center for Plant Conservation (CPC) has several recommendations for best reintroduction practices (Maschinski et al. 2012). One of these is to choose a recipient site with consideration of landscape characteristics (e.g., topography, ecosystem dynamics). A second recommendation is to use transplants of the largest size possible. However, for *Cirsium pitcheri* (Pitcher's thistle), there is a limit to the benefit of transplant size as previous data shows that larger transplants have lower fecundity due to immediate flowering (Bell et al. 2003). Therefore, transplant size may directly affect survivorship and population establishment (Davies et al. 1999). Long-term demographic studies can indicate whether microsite variation affects population growth and persistence and thus influences future management efforts (Maschinski et al. 2012).

In this study, we evaluate effects of transplant size and microsite characteristics on restoration success of *C. pitcheri*, federally listed as threatened (Harrison 1988). This species is threatened by habitat loss as well as by ecological processes of the human-altered shoreline. Recovery-planning objectives for this species include the establishment of a viable population in former habitat in Illinois, USA (Bowles et al. 1993). Our goals included determining effects of 1) microsite characteristics, slope, elevation, and aspect, on first-year survival; 2) initial transplant size on second-year growth of *C. pitcheri* transplants using a 10-year dataset. We then tested predictions that we developed from these results on additional specimens of *C. pitcheri* planted in 2013.

## Methods

### Organism and Study System

*Cirsium pitcheri* inhabits dynamic beach and open sand dunes of the western Great Lakes shorelines where it colonizes disturbance patches. These plants are poor competitors, and populations decline slowly as vegetation



**Figure 1. Study population in reference to potential habitat located on the western coast of Lake Michigan at Illinois Beach State Park in northeastern Illinois, US, approximately 70 kilometers north of Chicago, (87°29' W, 42°26' N).**

succession advances (McEachern 1992, Loveless 1984). As a monocarpic perennial, *C. pitcheri* grows for five to eight years and dies after one reproductive episode (Loveless 1984). *Cirsium pitcheri* plant size, measured by root-crown diameter (cm), can be used to predict survival and flowering as this species must reach a size threshold before flowering (Bell et al. 2002). Initially, we measured root-crown diameter directly along with leaf number and longest leaf length and found significant correlation between root-crown diameter and leaf-area index (product of leaf number and longest leaf length [Bell et al. 2013, supplementary material]). To reduce damage to the plant, in 1998 we began estimating root-crown diameter via the regression equation below (Equation 1) using leaf-area index.

$$\text{Crown diameter} = 0.05 + (0.033 \times \text{leaf number}) + (0.014 \times \text{length of longest leaf})$$

### Planting Strategy and Experimental Design

**1991–2000 Initial Reintroduction.** The restoration site is a low, narrow, ridge-and-swale sand deposit that extends for over 20 kilometers along the west coast of Lake Michigan at Illinois Beach State Park, Illinois, US. The study species was extirpated from this area circa 1920 (Figure 1;

**Table 1.** Number of *Cirsium pitcheri* transplants each year at Illinois Beach State Park, Illinois, US, along with first-year-survival rates and minimum (min) and maximum (max) slopes and elevation.

Year	# of Transplants	First-year Survival	Elevation (m)		Slope	
			MIN	MAX	MIN	MAX
1991	81	33.3%	178.10	179.79	0.30	16.35
1992	8	50.0%	179.15	180.30	7.55	18.47
1993	113	37.0%	177.90	179.79	0.70	16.35
1994	24	34.8%	179.44	179.99	3.34	12.75
1995	109	22.5%	178.23	180.24	0.22	18.56
1996	175	6.9%	178.60	181.03	0.35	20.94
1997	94	68.5%	178.31	179.74	1.08	17.94
1998	14	50.0%	179.08	179.61	12.47	12.74
1999	122	23.6%	178.00	181.02	0.31	24.40
2000	113	24.5%	178.37	180.76	0.76	21.40
2013	120	95.0%	178.15	179.88	0.35	23.73

Bowles et al. 1993). *Cirsium pitcheri* habitats at Illinois Beach include grass-dominated, north-south trending foredune, secondary dunes, and adjacent dune fields ranging in height from 178–180 m (McEachern et al. 1994). Dune habitat extends inland up to 100 m with greater than 75 percent open sand in patches with a shrub matrix (Bowles et al. 1993). At Illinois Beach, secondary dunes were chosen as optimum habitat since they were protected from shoreline erosion and human impact (Bowles et al. 1993). Reintroductions began in 1991 with 853 *C. pitcheri* greenhouse-grown plants transplanted in multiple cohorts over a ten-year period (Bowles and McBride 1996, Bell et al. 2003).

Plants were grown from seed using a potting mixture composed of 90% torpedo sand and 10% silt loam (Bowles et al. 1993). Seeds were collected by permit in 1990 from northern Indiana, southern Wisconsin, and southern Michigan (Bowles and McBride 1996). We planted *C. pitcheri* either as first-year seedlings in the fall or overwintered them and planted them in the spring before second-year growth was initiated (Bowles and McBride 1996). Each year, depending on the number available, transplants were planted across the suitable dune habitat in either circular plots or transects extending east from a north-south baseline (Bowles et al. 1993). We created a total of 39 plots, separated by at least 10 m each, many of which involved a single transplant event. *Cirsium pitcheri* was transplanted primarily on east and west facing slopes (Figure S1). Each year the locations of transplants varied along elevation and slope gradients (Table 1; Bell et al. 2013).

All plants were tagged with a unique identifier and mapped relative to permanent transects or plot markers via distance and azimuth. In 2000, sub-meter GPS (global positioning system; Trimble TSC1) locations of permanent markers were recorded and mapped locations from previous years were converted to UTM (Universal Transverse Mercator) units. The first natural recruit was produced in 1994, and the last transplant was recorded in 2005 (Bell et al. 2013).

Demographic monitoring occurred every year in August in which we located all plants and recorded survival status, root-crown diameter, leaf number, and the length of the longest leaf. Plants were also classified into one of three stages: seedlings (first-year plants with cotyledons), juveniles (non-flowering vegetation plants), and flowering. In 2006, this site was remotely sensed, creating a high-resolution digital elevation model (DEM; resolution 0.25 m). From this DEM we extracted the associated GIS data (elevation, slope, and aspect) for each plant using ArcMap (v. 10.3, Environmental Systems Research Institute, Redlands CA). Elevation ranged from 177.9 to 181.0 m, slope ranged from 0° (flat) to 23°, and aspect ranged from 0–360°.

*2013 Additional Plantings.* In 2013 we initiated additional plantings north of the Dead River as an experiment to test first-year-transplant survival rather than augmenting the existing *C. pitcheri* populations, which primarily remained south of the Dead River (Figure 1). This was primarily due to the fact that seeds of the exact population origins were no longer available (the originals came from an *ex-situ* experimental planting with mixed heritage from Wisconsin and Michigan.) These new plants were overwintered under greenhouse conditions and planted in Spring 2013. We distributed 120 *C. pitcheri* plants evenly among four plots (100–200 m<sup>2</sup>) that represented variation in elevation, slope, and aspect (Table 1; Figure S2). For each plant, we attached a unique identifier tag and recorded sub-meter GPS location (Trimble GeoXH). In 2014 and 2015 we again located plants and recorded survival status, stage, leaf number, and the length of the longest leaf. We also extracted slope, elevation, and aspect for each plant as noted above.

### Statistical Analysis

To determine how microsite variables influenced first-year-transplant success, we fitted general linear models (GLMs) and generalized linear mixed models (GLMMs) to first-year-survival and -growth data from the ten-year data set (1991–2000). We measured growth as the difference

**Table 2. Generalized linear mixed models associated with *Cirsium pitcheri* first-year survival of plants transplanted from 1991–2000 with year as a random variable. Predictor variables include slope, elevation, aspect, and root-crown diameter (CD) at time of transplant. Model ranks are based on lowest Akaike’s information criterion (AIC<sub>c</sub>).**

	$K^a$	AIC <sub>c</sub>	$\Delta AIC_c$	$W_i^b$	$\mathcal{L}^c$
CD	3	689.56	0	0.82	-341.76
Global	6	692.56	3	0.18	-340.22
Null	2	734.69	45.13	0.00	-365.33
Elevation	3	733.29	45.74	0.00	-364.63
Aspect	3	735.56	46.00	0.00	-364.76
Slope	3	736.59	47.03	0.00	-365.28

<sup>a</sup> number of parameters

<sup>b</sup> Akaike weight

<sup>c</sup> Log likelihood

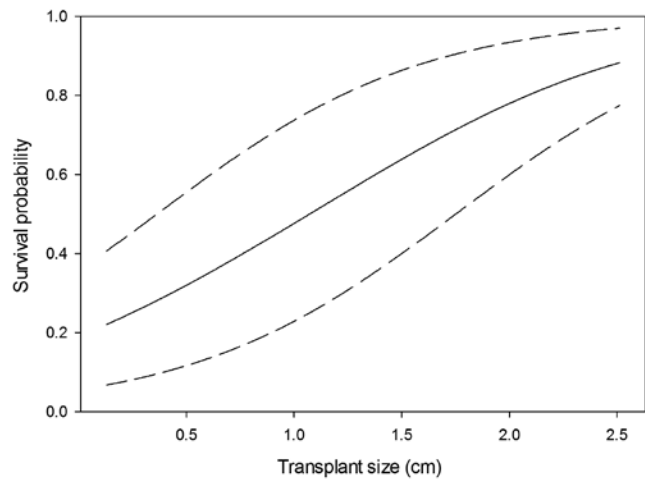
in root-crown diameter at the time of transplanting from that of the following year. We used information-theoretic approaches to infer differences between multiple models (Burnham and Anderson 2002). We developed a global model using all variables (slope, elevation, aspect, and initial transplant-root-crown diameter). We also ran a null model (intercept only) as a reference for assessing model importance (Anderson 2008). For all models, year was included as a random variable to control for year effects. Our complete set of candidate models included the null, global, and each independent landscape variable separately. We again used general linear models (GLMs) and generalized linear mixed models (GLMMs) for the 2013 data to test the predictions of microsite effects on first-year-survival and -growth data including global, null, and individual landscape variables.

We evaluated models using Akaike’s information criterion (AIC<sub>c</sub>) adjusted for small sample sizes (Burnham and Anderson 2002). All analyses were conducted in R v. 3.1.1 (R Foundation for Statistical Computing, Vienna, Austria). For mixed models, continuous predictors were scaled using the scale function which allowed for model convergence and adjustment of scale to match that of predictor variables. Functions used are available in the base and lme4 library (v 1.0–5). Lastly, we correlated first-year survival to monthly precipitation and temperature averages (NOAA 2008) to identify any role weather may have played in first-year-transplant survival.

## Results

### 1991–2000 Initial Reintroduction

From 1991 to 2000, *C. pitcheri* first-year-transplant survival ranged from 6.8% to 68.4% ( $m = 0.351$ ,  $s = 0.166$ ). The best fitting model for survival indicates that plant root-crown diameter at the time of transplantation affects first-year survival (Table 2), and higher probabilities of survival are



**Figure 2. The relationship between size of root-crown diameter at time of transplanting and survival probability of *Cirsium pitcheri* plants at Illinois Beach State Park, 1991–2000.**

**Table 3. General linear mixed models associated with *Cirsium pitcheri* first-year plant growth of plants transplanted from 1991–2000 with year as a random variable. Predictor variables include slope, elevation, aspect, and root-crown diameter (CD) at time of transplant. Model ranks are based on lowest Akaike’s information criterion (AIC<sub>c</sub>).**

	$K^a$	AIC <sub>c</sub>	$\Delta AIC_c$	$W_i^b$	$\mathcal{L}^c$
Global	7	42.85	0	1.0	-14.13
CD	4	61.78	18.93	0.0	-26.79
Elevation	4	74.21	31.36	0.0	-33.00
Null	3	97.15	54.51	0.0	-45.52
Slope	4	98.61	55.76	0.0	-45.20
Aspect	4	99.23	56.38	0.0	45.51

<sup>a</sup> number of parameters

<sup>b</sup> Akaike weight

<sup>c</sup> Log likelihood

associated with larger root-crown diameters (Figure 2). The model with root-crown diameter as the predictor variable was the best fit model with a  $\Delta AIC_c < 2$ , with an 80% of the Akaike weight meaning there is no model selection uncertainty. For plant growth, the strongest model incorporated all microsite variables (elevation, aspect, and slope as well as initial root-crown diameter) with an Akaike weight of 100% (Table 3). Higher elevation predicted increased plant size (Figure 3A), whereas increased slope predicted a decrease to smaller plant size (Figure 3B). There didn’t appear to be a difference in plant growth based on the direction of the slope (Figure S3).

### 2013 Additional Planting

When model predictions were tested with the 2013 data, we found that root-crown diameter did not predict first-year survival of *C. pitcheri* plants. Our model-set included model-selection uncertainty with three models having a

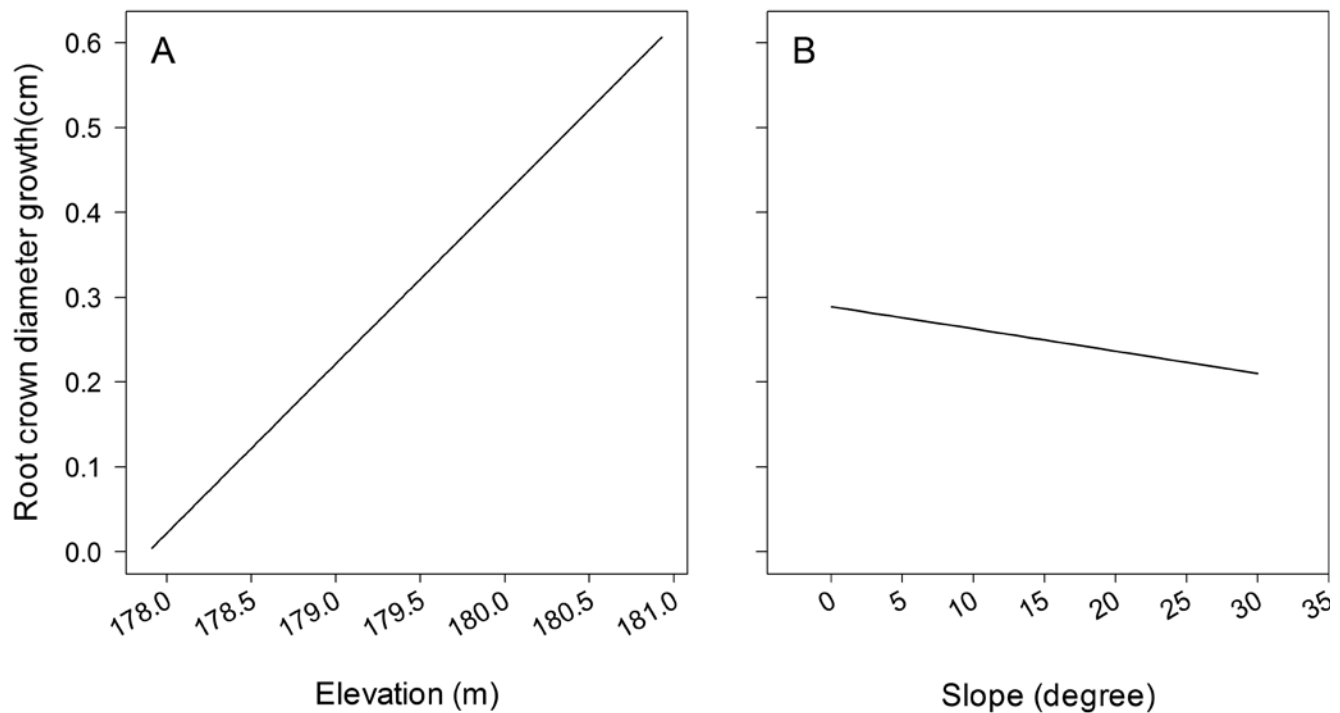


Figure 3. The relationship between A) elevation, B) slope, and predicted second year root-crown diameter growth for *Cirsium pitcheri* plants at Illinois Beach State Park, 1991–2000.

$\Delta AIC_c < 2$ , one of which was the null model (Table 4). This uncertainty may be a consequence of the 2013 planting yielding a 95% survival rate. First-year survival appears to be highly correlated with average monthly temperature for May (Linear regression;  $R^2 = 0.757$ ,  $p < 0.001$ ). *Cirsium pitcheri* plant growth, however, was associated with the plant size at the time of transplantation (Table 5).

## Discussion

Our study indicates how an experimental approach to transplanting may help guide restoration. This long-term study demonstrated that propagule size and microsite topographic variables (e.g., slope, elevation) significantly

affected the transplant success of *C. pitcheri*. First-year survival increased with transplant size, and the characteristics of plant microsite influence plant growth. *C. pitcheri* planted at higher elevations grew larger in contrast to plants on steeper slopes, which were smaller the year after transplantation. From these results, we made the following predictions: 1) larger *C. pitcheri* plants were more likely to survive the first year; 2) higher elevation would lead to increased second-year-plant size; and 3) steeper slopes would result in smaller second-year plants. We then sought to test these hypotheses with additional transplants in 2013 to determine whether this previous knowledge could guide us to greater transplant survival.

Table 4. Generalized linear models associated with *Cirsium pitcheri* first-year plant survival of plants transplanted in 2013. Predictor variables include slope, elevation, aspect, and root-crown diameter (CD) at time of transplant. Model ranks are based on lowest Akaike's information criterion ( $AIC_c$ ).

	$K^a$	$AIC_c$	$\Delta AIC_c$	$W_i^b$	$\mathcal{L}^c$
Aspect	3	-42.83	0.00	0.40	24.52
Null	2	-41.83	1.00	0.24	22.96
Slope	3	-40.55	2.28	0.13	23.38
CD	3	-40.53	2.30	0.13	23.37
Elevation	3	-39.76	3.07	0.09	22.98
Global	6	-36.75	6.08	0.02	24.75

<sup>a</sup> number of parameters

<sup>b</sup> Akaike weight

<sup>c</sup> Log likelihood

Table 5. General linear models associated with *Cirsium pitcheri* first-year plant growth of plants transplanted in 2013. Predictor variables include slope, elevation, aspect, and root-crown diameter (CD) at time of transplant. Model ranks are based on lowest Akaike's information criterion ( $AIC_c$ ).

	$K^a$	$AIC_c$	$\Delta AIC_c$	$W_i^b$	$\mathcal{L}^c$
CD	3	-82.92	0	0.95	44.58
Global	6	-76.84	6.08	0.05	45.86
Aspect	3	-59.87	23.04	0.00	33.06
Null	2	-59.85	23.07	0.00	31.98
Slope	3	-59.47	23.45	0.00	32.86
Elevation	3	-57.84	25.08	0.00	32.04

<sup>a</sup> number of parameters

<sup>b</sup> Akaike weight

<sup>c</sup> Log likelihood

Our data show that with *C. pitcheri*, the probability of first-year survival is influenced by the size at transplantation. Larger grown plants apparently had greater resources (e.g., root-system reserves) which increased survival (Dollard and Carrington 2013). Therefore, in the context of a single reintroduction effort, the best option for success may be increasing plant size to ensure the greater survival. However, there may be demographic tradeoffs to consider among survival, growth, and fecundity of plants that have different reproductive strategies. For example, although larger *C. pitcheri* plants have a higher survival rate when planted, earlier work shows that larger transplants that have accumulated threshold levels of root reserves may flower in the immediate planting year, and may have lower fecundity as a result (Bell et al. 2003). While these larger transplants may have reached the “threshold” needed to flower, they produce fewer flower heads and, in turn, fewer seeds (S. Halsey, unpublished data). Therefore, maximizing transplant size may result in short-term restoration success but will reduce population growth and viability. Because *C. pitcheri* is monocarpic, lower fecundity cannot be remedied, whereas polycarpic species may recover greater fecundity in subsequent reproductive years. As a result, increasing plant size to a maximum that is still below the flowering-threshold size may provide the best chance to overcome the first barrier to restoration success, initial survival. For *C. pitcheri* plants that do not flower immediately, further growth, and ultimately the persistence of the individual, depends on microsite characteristics.

Successful establishment of plants depends on site suitability (Primack 1996), with optimum sites promoting plant growth and survivorship. In dune habitats, plants at higher elevations grew larger than at lower elevation whereas plants on steeper slopes growth at a slower rate and in some cases were smaller in the year following transplantation. There appeared to be no noticeable effect due to the direction of the slope on either first-year-transplant survival or second-year-plant growth. Responses of *C. pitcheri* to dune topography may be related to environmental disturbances such as sand burial that results from either wind, waves or recreational activities (Maun et al. 1996). Sand burial can have varying effects on *C. pitcheri* emergence and survival, with small amounts of burial stimulating plant growth but larger amounts inhibiting it (Rowland and Maun 2001). At higher elevations, plants are less likely to be buried by sand, yet steeper slopes may result in more unstable sand accretion, increasing the chances of sand burial or erosion and perhaps exposure of roots. Steep slopes are also likely better drained, producing a more arid environment in which lack of moisture might explain slower growth.

Rare plant reintroductions are an essential component of conservation biology (Dobson et al. 1997, Maschinski and Haskins 2012). However, despite their popularity,

reintroductions are rarely 100% successful (Allen 1994, Albrecht et al. 2011, Godefroid et al. 2011). This is shown by the variability of survival for the 853 plants we transplanted over the course of ten years with an overall total of 48% plants surviving the first year. In 2013, the 95% survival rate was unprecedented and seemed to be correlated with a high average monthly May temperature. A critical metric of restoration efforts is short term survival, with the most commonly reported assessment of reintroduction success being first-generation survival and establishment (White 1996). Survival of transplanted individuals is only a preliminary step (Primack 1996) with a critical subsequent measure of a successful restoration being the ability of the population to persist and reproduce (Godefroid et al. 2011). Plants must survive the first year for this to happen, and greater survival results in greater potential for population persistence (Godefroid et al. 2011, Albrecht and Maschinski 2012).

Restoration efforts should focus on ensuring that habitat is suitable for the full completion of a plant’s lifecycle (Bowles et al. 2015). This would involve following the plants throughout their lifecycles to determine whether the conditions that enabled the survival of transplants allow for the successful production of offspring. A rapid test would be the planting of seeds as those results may differ from the planting of juveniles. Regardless, for long-lived species, identifying optimal habitat requires long-term studies (Maschinski et al. 2012, Lanno and Sammul 2014).

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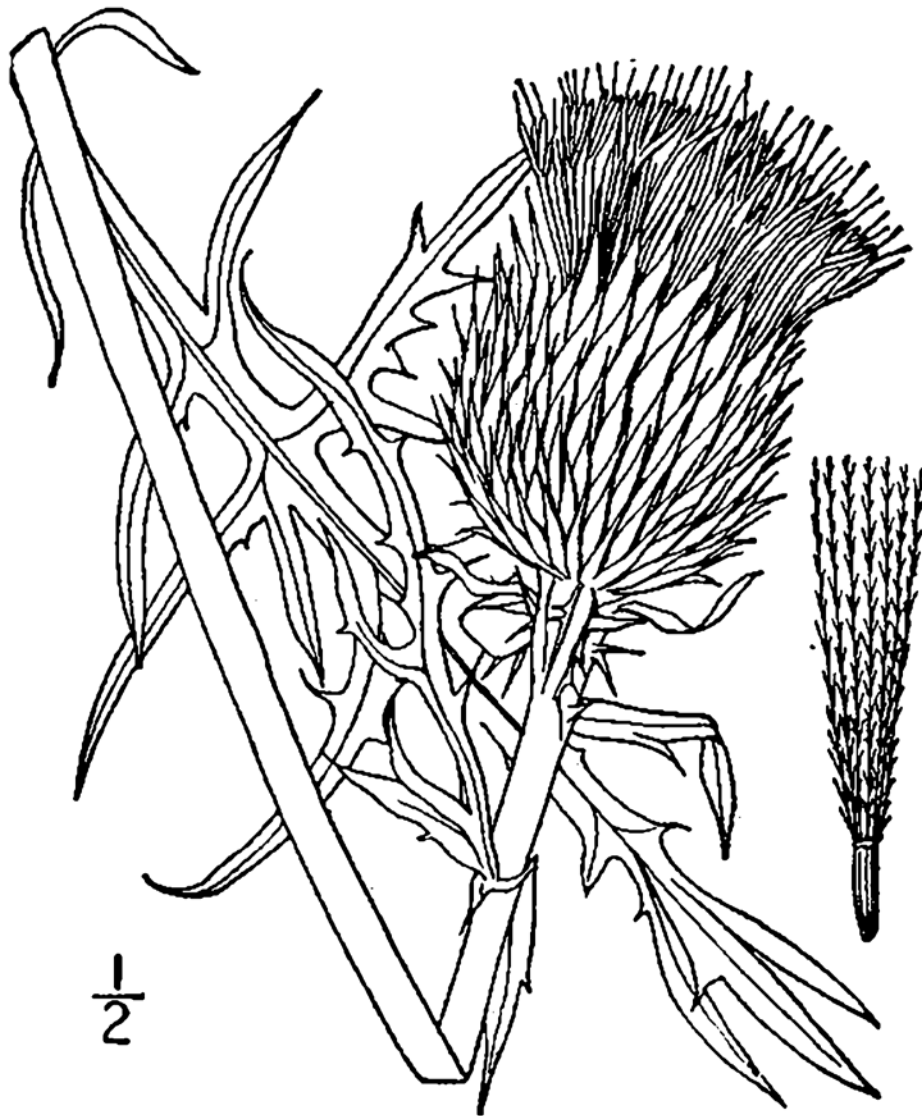
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