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Abstract

Cirsium pitcheri (Torr. ex Eaton) Torr. & A. Gray (Pitcher's thistle) is a threatened herbaceous plant endemic to sand dune ecosystems along Lakes Huron, Michigan, and Superior in North America. Habitat for this plant is limited to active dunes with moving sand. I observed floral visitors of *C. pitcheri* in Indiana Dunes National Lakeshore and Indiana Dunes State Park, and calculated frequency and density of visitor families. Additionally, I tested for relationships between visitor counts and previous growing season mean temperature and precipitation. Formicidae, Anthomyiidae, and Cecidomyiidae were the most frequent families. However, Apidae was the only family correlated with the number of subsequent *C. pitcheri* seedlings. Counts of mean visitors per plant were different between years, with 2013 being the lowest. These values were related to previous growing season precipitation, which was lowest in 2012 due to a widespread severe drought. There was clear depression of floral visitor frequency and density following the 2012 drought, but that was short-lived and subsequent years displayed recovery of visitor numbers. Many of the floral visitors of *C. pitcheri* are likely feeding on nectar, pollen, and vegetative structures, and providing minimal, if any, pollination benefit. However, families such as Apidae and Halictidae carry visible pollen loads between multiple individual plants. Pollinator augmentation with these families may benefit *C. pitcheri* reproduction, especially following years of drought.

Keywords: Apidae, Pollinators, Pitcher's thistle, sand dunes

Open sand dune ecosystems along the Great Lakes shoreline in North America are exceedingly sensitive to various anthropogenic disturbances including residential and recreational development and activities (van Dijk and Vink 2005). This sensitivity is due to the existing repeated natural disturbances to which specialized plant communities have developed composed of species adapted to exploit limited resources (Moreno-Casasola 1986, Maun 1998, Maun and Perumal 1999, Lichter 2000, Bach 2001). When that continual sand movement related to dune development and maintenance is altered or halted, new species can colonize the area displacing those native, specialized plants (Marshall et al. 2008). Successful ecological restoration efforts have focused on mimicking natural succession within dunes (Emery and Rudgers 2009). With alteration of Great Lakes sand dune ecosystems comes the alteration of limited habitat for rare species that only occur in those dunes.

One of those rare plant species is *Cirsium pitcheri* (Torr. ex Eaton) Torr. & A. Gray (Asteraceae, Pitcher's thistle), which is endemic to the sand dune ecosystems along the Great Lakes. The range for *C. pitcheri* includes dunes along Lakes Huron, Michigan, and Superior, in Indiana, Michigan, Wisconsin, USA, and Ontario, Canada (Voss 1996, Higman and Penskar 1999, Indiana Department of Natural Resources 2007, Wisconsin Department of Natural Resources 2015, Global Biodiversity Information Facility 2016). Historically, *C. pitcheri* did occur along the Lake Michigan shoreline in Illinois, USA, but those populations were extirpated as a result of land development and have subsequently been reintroduced (United States Fish and Wildlife Service 2001, Illinois Endangered Species Protection Board 2015). As a result of the rarity of *C. pitcheri* and its necessary habitat, as well as the sensitivity of both the species and habitat, the United States Fish and Wildlife Service listed *C. pitcheri* as a threatened species (i.e. likely to become endangered within the foreseeable future) in 1988 (United States Fish and Wildlife Service 2001).

Cirsium pitcheri has an extended maturation period (5–8 years) between seedling establishment and a single flowering event, after which the plant senesces (United States Fish and Wildlife Service 2002). Typically, *C. pitcheri* occurs in areas with increased bare sand (less neighboring vegetation)

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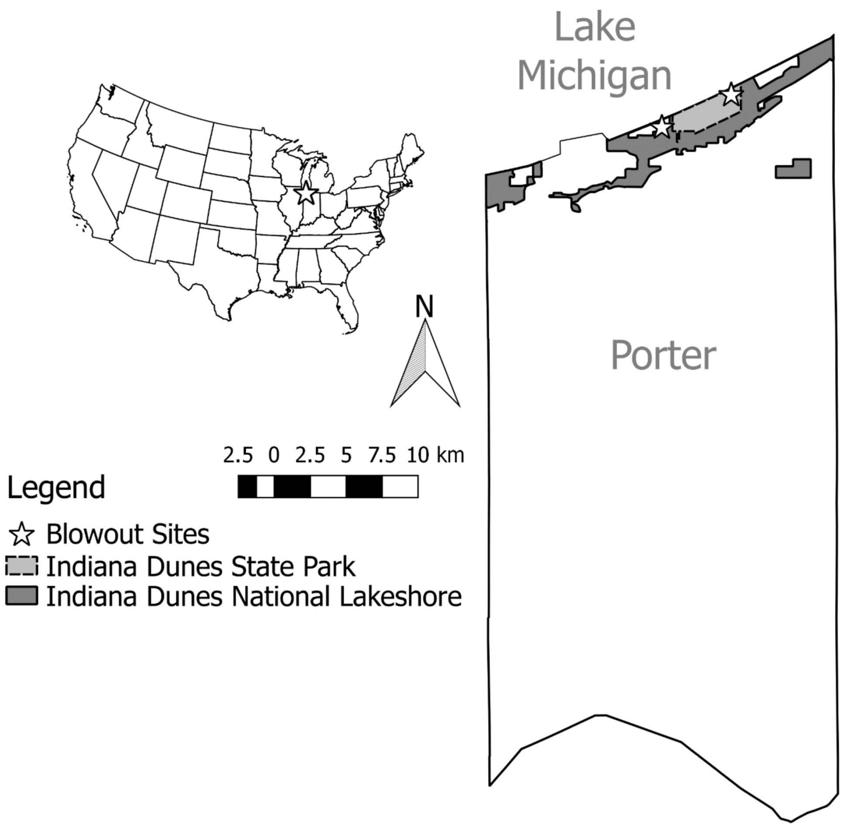

Figure 1. Indiana Dunes National Lakeshore and Indiana Dunes State Park with blowout locations used for *Cirsium pitcheri* surveys in Porter County, Indiana, USA.

compared to more stabilized areas and in dune blowouts, as a result of active sand movement (Bowles et al. 1990, McEachern et al. 1994, Marshall 2014, Jolls et al. 2015). Consequently, burial and cold stratification are required for successful *C. pitcheri* seed germination (i.e. Chen and Maun 1998, 1999; Hamzé and Jolls 2000). As such, not only is *C. pitcheri* limited in distribution to Great Lakes dunes, but it is also limited in distribution within those rare and sensitive ecosystems (Higman and Penskar 1999, Marshall 2014). Floral and seed herbivory add to limitations in distribution (Havens et al. 2012, Marshall 2013). Patterns of *C. pitcheri* occurrence within dunes are likely also limited by seed dispersing within 1.5–4 m from the parental plant and seed bank persisting two years maximum (i.e. Keddy and Keddy 1984, Bowles et al. 1993, Rowland and Maun 2001, Jolls et al. 2015).

By quantifying insect visitors to flowers and their effectiveness in pollination of *C. pitcheri*, we can better understand the ecological importance of these families in *C. pitcheri* populations. The objectives of this study were to 1) survey the insect visitors of *C. pitcheri* within established populations in Indiana Dunes National Lakeshore and Indiana Dunes State Park, 2) quantify the subsequent seedling presence as a surrogate for seed viability, 3) identify relationships between insect visitation, seedling counts, and local growing season weather, and 4) test the hypotheses that weather patterns influence floral visitation and that certain insect taxa are more important to the success of *C. pitcheri* reproduction.

Methods

Indiana Dunes National Lakeshore and Indiana Dunes State Park (henceforth IDNL and IDSP, respectively) are adjacent protected areas along Lake Michigan in Indiana, USA (Fig. 1). IDNL is managed by the National Park Service and covers approximately 24 km of shoreline and 6,070

Table 1. Insect families observed visiting *Cirsium pitcheri* individual plants during 2012-2015 and calculated frequencies (total number of plants visited by a family), densities (number of individuals per plant [N = 61]), and correlation between number of individuals observed and subsequent year *C. pitcheri* seedling count within 4 m of focal survey plants. Asterisk (*) indicates significant p-value.

Order	Family	Frequency	Density	r	p
Coleoptera	Chrysomelidae	11	0.26	–0.03	0.795
	Curculionidae	10	0.25	–0.17	0.199
	Melyridae	8	0.43	–0.19	0.139
	Scarabaeidae	1	0.02	–0.12	0.360
Diptera	Anthomyiidae	24	0.90	0.10	0.449
	Calliphoridae	2	0.03	0.05	0.702
	Cecidomyiidae	22	0.67	–0.06	0.657
	Syrphidae	15	0.89	–0.17	0.186
Hemiptera	Cicadellidae	1	0.02	0.05	0.704
	Pentatomidae	2	0.03	0.04	0.768
Hymenoptera	Apidae	16	0.79	0.34	0.007*
	Formicidae	25	1.69	–0.11	0.409
	Halictidae	14	0.41	–0.10	0.463
Orthoptera	Acrididae	1	0.02	0.17	0.181

ha in area. IDSP is managed by the Indiana Department of Natural Resources and covers approximately 5 km of shoreline and 883 ha in area. Both properties include active sand dunes and hardwood forests.

Populations of *C. pitcheri* were selected, one in each IDNL and IDSP, within two well established blowout features (Fig. 1). Each year (2012–2015), ten flowering *C. pitcheri* individuals were selected randomly from the population. If ten individuals could not be located, then all flowering individuals were selected. During July of each year, selected *C. pitcheri* individuals were observed for 10 minutes on two consecutive days during a 4-hour time bracket centered on solar noon. All insect floral visitors were identified to family. Prior to observation, all flowering *C. pitcheri* individuals were counted within a 4 m radius plot centered on the selected individual. In subsequent years (including 2016 for the 2015 focal flowers), first-year seedlings were counted within a 4 m radius plot centered on each focal flower location, which is the maximum distance seedlings are typically found from the parent plant (Keddy and Keddy 1984). Frequency (number of observed plants visited) and density (number of individuals visiting each observed plant) were calculated for each family observed. Previous year's weather was summarized for the growing season (March–September) as total precipitation and mean temperature (National Oceanic and Atmospheric Administration 2016).

T-test was used to compare mean total number of *C. pitcheri* flowering and first year seedlings between IDNL and IDSP within the 4 m radius plots. Simple linear regression was used to test for relationships between density of flowering individuals within 4 m radius of focal plant and number of floral visitors. Analysis of variance (ANOVA) with Tukey's HSD as a post-hoc test was used to compare mean count of visitors per plant between survey years. Simple linear regression was used to test for relationships between mean count of visitors per plant and previous year growing season weather (total precipitation and mean temperature). Pearson correlation was used to test for relationships between insect family counts at the focal plant and subsequent year *C. pitcheri* seedling counts.

Results

At IDNL, 10 flowering individuals were observed in 2012, 2013, and 2014. However, no plants were included at IDNL in 2015 due to permit application delays. At IDSP, 10 flowering individuals were observed in 2012, six in 2013, nine in 2014, and six in 2015. Density of flowering individuals within a 4 m radius plot was significantly greater in IDNL compared to IDSP (2.6 [SE 0.8] vs. 0.8 [SE 0.2]; $t = 2.27$; df = 59; $P = 0.014$). However, there was no difference in count of first year seedlings within 4 m of the observation plant location between parks ($t = 1.21$; df = 59; $P = 0.232$).

A total of 14 insect families were observed visiting *C. pitcheri* plants during survey years (Table 1, Fig. 2). While IDNL and IDSP had different densities of flowering *C. pitcheri* individuals, the number of floral visitors was not related to flowering density ($F = 1.44$; df = 1, 59; $P = 0.235$, $R^2 = 0.02$). Mean count of insect visitors per plant was significantly different between years, with

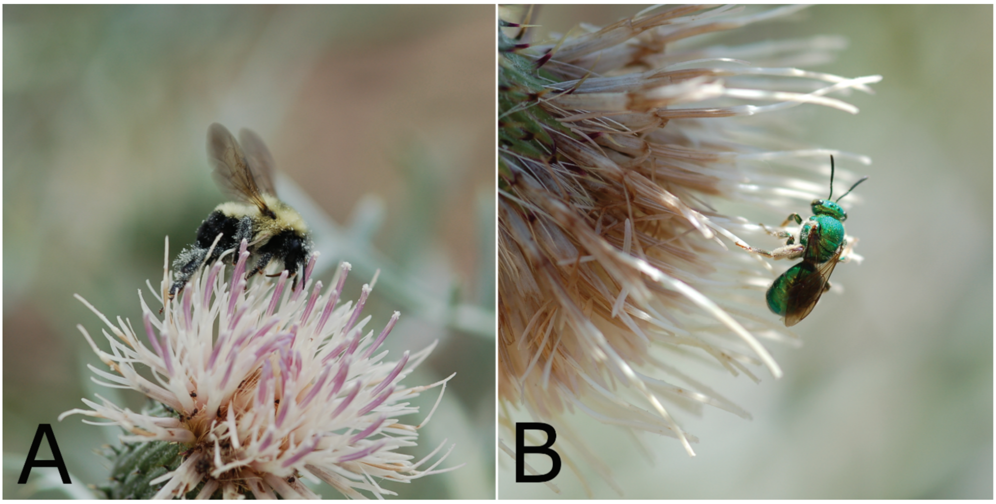

Figure 2. Example of floral visitors on *Cirsium pitcheri*. A: Apidae. B: Halictidae.

2013 having the fewest visitors (Fig. 3A). In 2013, Anthomyiidae, Apidae, and Calliphoridae had only one occurrence. The other families occurring in 2013 included Cecidomyiidae, Chrysomelidae, Curculionidae, Formicidae, and Halictidae (6, 4, 4, 25, 5 occurrences each, respectively). Mean visits per plant was not significantly related to previous growing season mean temperature ($F = 3.02$; df = 1,2; $P = 0.225$). However, mean visits per plant was significantly related to previous growing season total precipitation (Fig. 3B). Of the observed families, only Apidae counts were significantly correlated with subsequent year seedling counts (Table 1). All observed Apidae individuals were *Bombus* spp.

Discussion

Habitat for *C. pitcheri* is limited to sensitive sand dune ecosystems along Lakes Huron, Michigan, and Superior, in North America (Voss 1996). Within IDNL and

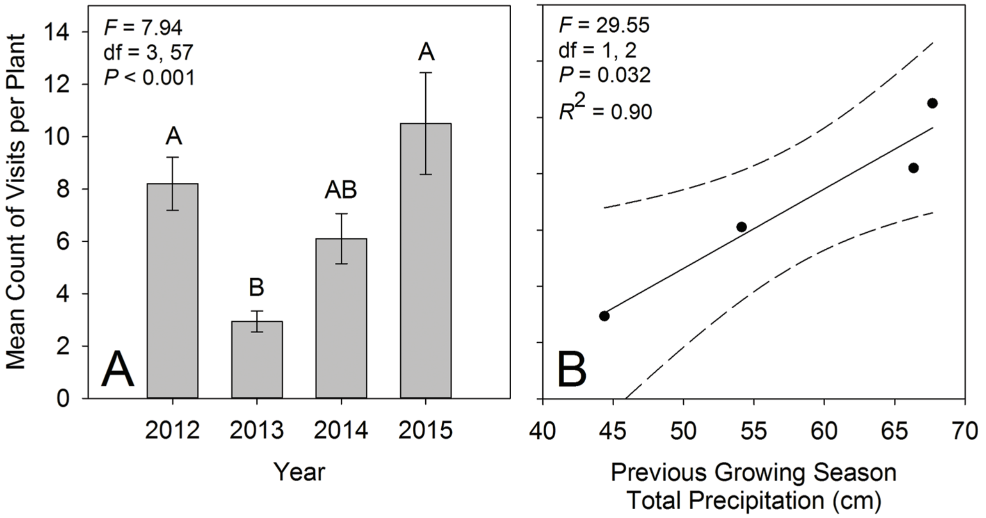

Figure 3. A: Mean count of insect visits per *Cirsium pitcheri* plants between survey years. Error bars represent standard error. Unique letters represent significant differences between years with Tukey's HSD. B: Simple linear regression of mean count of visits per *C. pitcheri* plants by total precipitation from previous growing season. Dashed lines represent 95% confidence intervals.

IDSP, as with other dune systems in the Great Lakes region, *C. pitcheri* is limited to blowout features with areas of active disturbance (Bowles et al. 1990, McEachern et al. 1994, Marshall 2014, Jolls et al. 2015). While not quantified for this study, IDNL areas surveyed had lower overall plant cover compared to IDSP areas surveyed, which likely explains the greater density of flowering neighbors around observational plants in IDNL.

Seed predation, by both vertebrate and invertebrate animals, and floral feeding by agricultural pests likely have significant impact on *C. pitcheri* reproduction success (Loveless 1984, Phillips and Maun 1996, Havens et al. 2012, Marshall 2013). Also, mortality rates are relatively high in juvenile stages (D'Ulisse and Maun 1996). Adding to these obstacles, many of the insect families observed in this study have rather limited effectiveness in pollination. Neither the two families with the greatest frequencies and densities (Formicidae and Anthomyiidae), nor the third most frequent family (Cecidomyiidae), are known to be effective pollinators. Formicidae can negatively affect pollination efficacy and has been omitted in counts from previous studies regarding *C. pitcheri* pollination (Beattie et al. 1984, Baskett et al. 2011). Anthomyiidae and Cecidomyiidae are common fly families and have been found as stem and flower head feeders in other thistles (Gassman and Kok 2002). Within the same family as *C. pitcheri* (Asteraceae), Anthomyiidae individuals cause feeding damage in flowers (Anderson 1996). Additionally, Cecidomyiidae are known to frequent small tubular flowers found in Asteraceae (Larson et al. 2001). While these three families had high frequency and density values, they were not significantly correlated with subsequent year seedlings, likely because of their lack of pollination activity.

Apidae was the only family that was significantly correlated with subsequent year seedling counts. This family carries obvious pollen loads and visibly move from *C. pitcheri* individual to individual (personal observations). While Halictidae visitation was not correlated with seedling counts, it is another family that carries visible pollen loads and moves directly between *C. pitcheri* individuals. Additionally, both of these families are known important pollinators in Asteraceae, including *C. pitcheri* (Olsen 1997, Theis et al. 2007, Baskett et al. 2011,).

During 2012, much of the United States, including the Great Lakes region, experienced a significant drought that had widespread impact on agricultural systems (Mallya et al. 2013). The effect of this drought on the floral visitors was clear in 2013. Subsequent years (2014 and 2015) illustrated recovery as the drought lessened in the region after 2012. The pattern of mean visitors per plant was not significantly related to the previous growing season mean temperature, but was linearly related to the mean precipitation of the previous growing season. Scarcity of Apidae (specifically *Bombus* spp.) in Europe has been observed and linked to drought conditions (Rasmont and Iserbyt 2012). Pollination success in *C. pitcheri* may be severely impacted following major drought events when floral visitor populations are suppressed.

In this study, I used seedling counts from the following year as a surrogate for pollination success. There is little persistent seed bank for *C. pitcheri* (Bowles et al. 1993). While the majority of new individuals establish as the result of germination from the previous year's seed crop, there is potential for a short-lived seed bank (Jolls et al. 2015). However, one limitation for this study was the higher density of other flowering *C. pitcheri* individuals at some locations at INDL compared to INSP, which would have added to the seed bank for the following spring germination. Because of the presence of other flowering *C. pitcheri* during the course of this study, the correlations between Apidae individuals and neighboring seedling establishment should be interpreted conservatively. Quantifying pollen loads carried by individual visitors would likely add a layer of confidence to measuring visitor roles in pollination. Additionally, pollen contamination rates from other species on visitors may also aid in measuring such roles. Collecting seed from senescing flowers following successful pollination would have also added to this measure. However, interfering with successful pollination (measuring pollen loads and contamination) or dispersal (collecting seeds) could pose an undue burden on an already rare population. Overall, these limitations add external factors that complicate the interpretation of pollinator visitation and seedling success in *C. pitcheri* (i.e. seed predation, mate densities, residual seed bank). However, pollinator visitation rates have some influence on seedling success and provides initial insight regarding the success of *C. pitcheri*.

Because of the limited distribution of suitable habitat and individuals within that suitable habitat, *C. pitcheri* may benefit from augmenting local pollinator communities. Lab rearing and releasing native pollinator species, specifically *Bombus* spp. in the family Apidae, may improve pollination success and subsequent seed production. Augmentation may be especially beneficial after drought years where naturally occurring floral visitors are suppressed.

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