THE ANTAGONISTIC AND MUTUALISTIC PLANT-INSECT INTERACTIONS OF PITCHER'S THISTLE (*CIRSIUM PITCHERI* [TORR. EX EAT.] TORR. & A. GRAY, ASTERACEAE), A FEDERALLY THREATENED GREAT LAKES DUNE AND COBBLE SHORE ENDEMIC PLANT

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Biological control is one of the tools used for integrated pest management of invasive plant species but it is not without risks to native plants. I researched the non-target impacts of the biological control agent, the seed head weevil *Larinus planus* (Coleoptera: Curculionidae) on the Great Lakes dune and cobble shore endemic threatened thistle, *Cirsium pitcheri* (Asteraceae). Pitcher's thistle is an herbaceous perennial monocarpic plant with no means of vegetative reproduction, relying solely on seed set for population persistence. The seed head weevil is univoltine and lays eggs in thistle heads. The developing larva chews the ovules or seeds before emerging as an adult to overwinter in leaf litter. I repeatedly surveyed Pitcher's thistle plants from three populations in northern lower Michigan for impacts. The insect oviposits on thistle heads from mid-June to early July, before *C. pitcheri* flowering. Heads that received oviposition were on average 12-14 mm in diameter. Approximately 32% of the 1,695 heads surveyed had oviposition. A subset of dissected heads had 56% weevil egg mortality. With weevil survival, the number of filled seeds was reduced by 62%. A generalized linear mixed binary logistic model reported date of oviposition and size of heads as significant predictors of oviposition on heads.

I tested the effectiveness of an organic insect deterrent, Surround® WP, in reducing impacts on *C. pitcheri*. The kaolin clay is mixed in water and then applied to the plant create a protective film after drying. I first needed to confirm that kaolin clay did not negatively impact pollinator visits, which are important for seed set. To test this, I performed simultaneous 10 min observations on kaolin clay treated plants and untreated plants. Kaolin clay did not deter insect visits, affect species richness of visiting insects or change the length of the visit. To test the effectiveness in deterring the weevil I applied either kaolin clay or a water control to pairs of heads at Petoskey State Park. The clay-treated heads had significantly fewer oviposition holes, and were less likely to have oviposition holes at all. There was no

significant difference in the mean number of chewed seeds between treatments; however, there were significantly more filled seeds in heads treated with kaolin clay than in water-treated heads. I recommend the application of kaolin clay to reproductive Pitcher's thistle plants mid-June to early July every 3-7 days on heads not yet flowering. Reduction of impact from *Larinus planus* is critical for conservation of this species and may also be important for the entire network of insect flower visitors in the dune ecosystem.

Pitcher's thistle flowers from late-June to early August. Many species of insect from several taxonomic orders visit Pitcher's thistle flower heads indicating a generalist pollination syndrome. Generalist plants are often important floral resources that maintain plant-pollinator network structure, potentially as keystone species. I hypothesized that Pitcher's thistle is an important floral resource for the flower-visiting insects during its flowering period. To test this hypothesis I performed insect visitor observations on all the insect pollinated plants in randomly selected plots in the dunes of Sturgeon Bay, Wilderness State Park, MI. *C. pitcheri* received more visits and had more visitor species than any other plant in the network by a large margin. I used R Bipartite package to calculate species-level network metrics such as species strength and weighted connectedness and betweenness. I consistently found Pitcher's thistle to have the highest scores. The index *d'* rated Pitcher's thistle as the most generalized, i.e., received the most insect visitors. *C. pitcheri* also had a disproportionate effect on the flower-visiting insect fauna, relative to the abundance of its floral resources (number of open flowers, number of plants observed). Other plants in plant-pollinator networks have been shown to be important, perhaps keystone species, using this technique of species-level network metrics. Pitcher's thistle is an important species for the plant-insect network of the dune ecosystem. Insects and the flowering plants they visit and should be prioritized for conservation.

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Dedicated to my many academic and professional mentors

for aiding and enriching my career in plant conservation.

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TABLE OF CONTENTS

LIST OF TABLES

species percentages were derived from the 59 total insect taxa in the network. Insect visits percentages were computed from the 600 total observed.

8. Table 8. Insect visitors and number of visits to *Cirsium pitcheri* in the Sturgeon Bay dune plant-insect flower visitor network surveyed during the 2015 *C. pitcheri* flowering season.
9. Table 9. Species level network indices for the Sturgeon Bay dune flowering plant-insect visitor network. The indices are normalized degree (ND), species strength (SS), betweenness (B), weighted betweenness (BW), closeness (C), weighted closeness (CW) and d'.

LIST OF FIGURES

7. Fig. 7. Mean (± SE) numbers of seeds per category of *Cirsium pitcheri* heads treated with water ($n = 45$) and kaolin clay ($n = 38$). The Potential Seed Set category is the sum of Unfilled/Flat, Filled and Chewed categories. There were significantly more filled seeds in kaolin clay treated heads than water treated heads. (*) $p < 0.05$.
8. Fig. 8. Phenology of flowering periods of Great Lakes dune insect pollinated plant species. Flowering period data from floras (Voss, 1972; Ownbey and Morley, 1991; Flora of North America Editorial Committee, 1993; Chadde, S. 2013; Fig. 3).
9. Fig. 9. The Sturgeon Bay dunes plant-insect visitor network as sampled during the *Cirsium pitcheri* flowering season in summer 2015. The right hand bars represent the insect visitors; the left represent the plants. Width of the respective bars depicts the total number of visits performed or number of visits received for insects and plants, respectively. The width of each gray bar shows the strength of the connection between each pair of plants and insects.
10. Fig. 10. Number of insect visits each species received relative to abundances of the A) the total number of open flowers and B) the total number of plants that were observed for insect visits in the dune network. The legend names correspond to the first two letters of the genus and specific epithet of the plant taxa.

CHAPTER 1: INTRODUCTION TO THE PITCHER'S THISTLE SYSTEM

Plants can contribute many ecosystem services in their respective habitats (Altieri, 1999; Díaz and Cabido, 2001). For example, bryophytes (mosses, hornworts and liverworts) are important colonizers on bare mineral soil, establishing organic soil layers (Jonnson and Esseen, 1998; Garibotti et al., 2011). The bean family (Fabaceae) is known for the ability of most members to fix nitrogen, a nutrient necessary for plant growth, promoting the growth of other species nearby (Spehn et al., 2005).

Even rare plants, those that have limited geographic distribution, local abundance, and habitat specificity (Rabinowitz, 1981), can offer important ecosystem services. There are 886 threatened and endangered plant species listed in the United States under the Endangered Species Act (50 U.S.C §17.12, 2014). An endangered species is an organism whose numbers are threatened to point of extinction "as a consequence of economic growth"; a threatened species is any species likely to become endangered in the near future (16 U.S.C. § 1531-1544, 1973). Some rare members of the moss genus *Sphagnum* (Sphagnaceae) can provide ecosystem services, including the hydrological roles of holding and slow release of water, as well as providing structural habitat for other taxa; these rare taxa function as keystone species for bogs and fens in North Carolina (Murdock, 1994). Many species of the genus *Sarracenia* (Sarraceniaceae) are threatened, yet these unique insect carnivorous plants can serve as an indicator species for ecosystem health and function in wetland systems (Jennings and Rhor, 2011). Bark, leaves and fruit of the marula tree (*Sclerocarya birrea* (A. Rich.) Hochst. subsp. *caffra* (Sond.) Kokwaro, Anacardiaceae) are important sources of food for a wide variety of mammal fauna including endangered elephants in South Africa. This tree thus serves as an important species for structuring the savannah ecosystem (Jacobs and Biggs, 2002). In agricultural ecosystems, diversity of pollinating arthropods relies directly on the assemblage of native angiosperm pollen and nectar resources (Issacs et al., 2009). The rare wetland perennial of Oregon, Washington, and British Columbia, *Grindelia integrifolia* DC (Asteraceae), has specialized pollination relationships with a few native bee species, increasing local insect diversity (Severns and Moldenke, 2010).

In turn, flowering plants depend on native pollinators for reproduction, even in low productivity ecosystems such as dunes. Riverine dunes in eastern Germany have a high level of endemism of plant and arthropod species. This ecosystem is highly dependent on wild bee species for pollination of native plants. Bee diversity serves as an indicator of dune restoration success (Exeler et al., 2009). *Eremosparton songoricum* (Litv.) Vass (Fabaceae) is a rare legume native to the sand dunes of the Gurbantunggut Desert in China. Although the plant has vegetative means of reproduction, sexual reproduction is dependent on only four native bee taxa. *E. songoricum* suffers from inbreeding depression and requires larger populations with healthy abundances of pollinators to maintain resilience (Shi et al., 2013). The Great Basin desert dune ecosystem in Utah (USA) is highly impacted by the use of off-road vehicles on plant species and their pollinators; to mitigate these impacts, land managers set aside areas for conservation purposes (Wilson et al., 2009).

The dependence of the dune flora on arthropod diversity and abundances can pose management challenges. Wilson et al. (2009) found that patches of conserved dunes had low similarity both to each other and to non-conserved patches, using measures of bee species richness and abundance. This limited similarity suggests that conservation efforts were not sufficient to preserve pollination services for the many endemic plants in the system. *Jacquemontia reclinata* House ex. Small (Convolvulaceae) is a member of the morning glory family restricted to the dunes of the coast of southeastern Florida. This habitat is increasingly fragmented by human development, restricting movement of native dune bees, flies and butterfly pollinators between plant populations. This plant species is dependent on native insects to transfer pollen for successful seed set. Increased habitat for pollinators and increased numbers of pollinators are recommended restoration efforts to increase *J. reclinata* population size (Pinto-Torres and Koptur, 2009).

The Great Lakes Basin of the United States and Canada has a wealth of unique habitats and globally significant biological diversity, including the world’s largest area of freshwater dunes. Habitats such as dunes and coastal shore wetlands support a high level of endemic and threatened species. Coastal shore habitat (including sand dunes) supports 30% of threatened animal and plant species found in the Great Lakes Basin (Rankin and Crispin, 1994). Coastal shore sand dunes support the Lake Huron locust (*Trimerotropis huroniana* Walker, Acrididae, Orthoptera) as well as the endemic plant species Houghton’s goldenrod (*Oligoneuron houghtonii* [Torr. & A.Gray ex A.Gray] G.L.Nesom, Asteraceae), Lake Huron tansy (*Tanacetum bipinnatum* (L.) Sch. Bip. ssp. *huronense* (Nutt.) Breitung, Asteraceae), and

Pitcher’s thistle (*Cirsium pitcheri* [Torr. ex Eaton] Torr. & A. Gray, Asteraceae), all restricted to the natural sand dunes of the Great Lakes (Michigan Natural Features Inventory, 2013).

Cirsium pitcheri is an herbaceous perennial monocarp native to the natural sand dunes of the western Great Lakes of USA and Canada (Lakes Superior, Michigan, Huron) (Voss, 1972). It typically grows for approximately 4-8 years in a vegetative rosette before bolting and setting 1-35 floral heads in June-August, then dying after seed set (monocarpic). *C. pitcheri* does not reproduce vegetatively and relies solely on seed set for reproduction (Keddy and Keddy, 1984; Hamzé and Jolls, 2000). Selfing can occur by pollinator movements within heads as well as the plant, but more seeds per head are produced with outcrossing (Loveless, 1984). The flowering plant is primarily pollinated by members of the genus *Bombus* and the bee family Halictidae (Hymenoptera), but visits from other solitary bees, butterflies, flies, and beetles have also been documented (Keddy and Keddy, 1984; Loveless, 1984; Baskett et al., 2011). This wide variety of insect visitors suggests *C. pitcheri* is a generalist and may function as an important floral resource in the Great Lakes natural dune ecosystem.

There have been many studies in the primary literature on various aspects of *C. pitcheri* ecology and life history, including genetics (Gauthier et al., 2010; Fant et al., 2013; Fant et al., 2014), restoration (Maun, 1997; Rowland and Maun, 2001; Baskett et al., 2011; Emery et al., 2013), herbivory (Phillips and Maun, 1996; Stanforth et al., 1997; Bevill et al., 1999; Rowland and Maun, 2001; Louda et al., 2005; Havens et al., 2012; Marshall, 2013), response to invasive species (Baskett et al., 2011; Leicht-Young and Pavlovic, 2012; Emery et al., 2013), germination (Chen and Maun, 1998; Chen and Maun, 1999; Hamzé and Jolls, 2000), habitat requirements (McEachern, 1992; Anwar et al., 1996; D'Ulisse and Maun, 1997; Rowland and Maun, 2001;Girdler and Radtke, 2006; Perumal and Maun, 2006; Marshall, 2014) and demography (Promaine, 1999; Havens et al., 2012; Jolls et al., 2015). Three significant works have examined pollination, seed set and seed predation (Keddy and Keddy, 1984; Loveless, 1984; Baskett et al., 2011).

In 1984, Keddy and Keddy sought to answer basic questions about *C. pitcheri* reproductive biology. They found the number of flowers and then seeds in a thistle inflorescence (head) are functions of head diameter. A single head can produce between 30-276 open flowers. The researchers also made pollinator observations and observed 10 species of insects in the orders Hymenoptera (bees), Diptera (flies) and Lepidoptera (butterflies). They also quantified the effects of a seed head predator, *Platyptila carduidactyla* Riley (Pterophoridae, Lepidoptera), the plume moth caterpillar. The females oviposit eggs in Pitcher's thistle heads and consume the ovules and developing seeds. Plants suffered 14-42% reduction in seed set per head (depending on grass or debris habitat) with the moth present compared to uninfected plants (Keddy and Keddy, 1984).

In 1984, Loveless examined *C. pitcheri* mating systems, reproductive biology and seed set. Bagging experiments showed partial self-compatibility in Pitcher's thistle, but higher levels of seed set resulted from heads that were treated with outcrossed pollen. Loveless (1984) followed pollinators through the dune landscape and noticed a pattern of differing visitation throughout the *C. pitcheri* blooming period. In early and late season observations, when fewer heads per plant were in flower, insects were more likely to visit heads between plants, increasing the chance of outcrossing. In middle season observations when more heads per plant were in flower, insects were more likely to visit heads within plants increasing the chance of selfing. Loveless observed the activity of 22 different species of insect in the orders Hymenoptera, Lepidoptera and Coleoptera (beetles). She also identified squirrels, deer, gold finches and several insects as seed predators impacting Pitcher's thistle seed set.

Baskett et al. (2011) offer the most recent study of *C. pitcheri* pollination. They examined the effect of two invasive plant species on *C. pitcheri* pollinator diversity and abundance by comparing plots before and after removal of invasives (restored plots). Plots with the invasives baby's breath (*Gysophilla paniculata* L., Caryophyllaceae) and spotted knapweed (*Centaurea stoebe* L., Asteraceae) had pollinator abundances that were five times higher than in the restored plots. Pollinator visits to *C. pitcheri*, however, were significantly higher in restored plots. Thus, the invasive plant species attracted more pollinator species to the plots, but increased the heterospecific pollen transferred to *C. pitcheri* and reduced visitation in general (Baskett et al., 2011). The threat of invasive plant species is one of the many factors that contribute to the federally threatened status of *C. pitcheri*.

Threats to *C. pitcheri* populations are numerous and extensive. These threats include 1) habitat loss and degradation due to anthropogenic development and noxious weed invasions (Basket et al., 2011), 2) low genetic diversity (Gauthier et al., 2010), 3) natural herbivory by gold finch, deer, squirrel, and insect herbivores (Loveless, 1984; Phillips and Maun, 1996; Bevill et al., 1999; Marshall, 2013), 4) low seed germination and establishment in the field (Hamzé and Jolls, 2000), and 5) potential range restriction from climate change (Vitt et al., 2010) in addition to declines associated with natural succession (Jolls et al., 2015). A new threat has emerged in the last decade: the seed head weevil, *Larinus planus* Fabricius (Coloptera, Cuculioniodae), an adventive (not documented to have been intentionally introduced to North America), non-native insect used for biological control, observed throughout most of the plant's range (Havens et al., 2012).

Larinus planus is a species of weevil native to Europe where it feeds on the tribe Cardueae (Cynareae) of the plant family Asteraceae (McClay, 1990). To oviposit, the fertile female will chew a tunnel through the bracts surrounding the head of flowers and lay an egg in the tunnel. She will then plug the tunnel with plant material, saliva and frass. The larvae eat the ovules and developing seeds of the flower head as they mature. The pupated weevils emerge as adults between late summer and early winter of the same year (Wheeler and Whitehead, 1985).

Much still needs to be known about the interaction between *C. pitcheri* and *L. planus*. Such interactions and resultant impacts are based in part on the temporal overlap of the reproductive plant and insect phenology. The importance of *C. pitcheri* to the dune ecosystem as a floral resource for insects is also unexplored. The effects of the loss of this endemic dune species on arthropod resources and the entire pollinator community has the potential for large, negative impact on these globally significant ecosystems.

Plant-pollinator mutualisms are argued to be among the most important global ecosystem services benefiting both humans and local biodiversity (Kearns et al., 1998). One way to analyze plant-pollinator interactions is through network analysis. Network analysis can evaluate the connections between nodes or individuals within an interconnected group, and the robustness of the network based on those connections (Buchannan, 2002). This approach has been used in ecology to 1) analyze food webs and predator-prey relationships (Winemiller, 1990), 2) analyze parasite-host and parasitoid relationships (Tylianakis et al., 2007), and 3) analyze plant-pollinator relationships (Bascompte and Jordano, 2007; Exeler et al., 2009; Olesen et al., 2002; Campos-Navarette et al., 2013).

CHAPTER 2: THE ANTAGONISTIC NON-TARGET IMPACTS OF A BIOLOGICAL CONTROL INSECT *LARINUS PLANUS* ON *CIRSIUM PITCHERI*, POTENTIAL CONTROL AND MANAGEMENT RECOMENDATIONS

ABSTRACT

Non-target impacts of the biocontrol insect *Larinus planus* have been documented on the threatened Great Lakes dune endemic *Cirsium pitcheri* since 2010. Much is still unknown about this interaction and what is necessary to prevent such unintended negative impacts. I intensively surveyed 44 plants at three field sites in northern lower Michigan to track phenology, factors that influence oviposition and impacts of this interaction. I also tested an organic kaolin clay crop protectant on pollinator visits to Pitcher's thistle and effectiveness in preventing *L. planus* impacts. I found that oviposition took place primarily before the *C. pitcheri* flowering season, mid-June to July. A generalized linear mixed binary logistic model revealed that date of oviposition and size of heads were significant factors in predicting oviposition. Heads were on average 12.5 ± 0.2 mm in diameter at the time of receiving the first oviposition hole, and the majority of heads were less than 15 mm in diameter. Of the 1,695 heads repeatedly surveyed, 32% received oviposition. A subsample of dissected and oviposited heads had 44% weevil survival in the heads to damage developing ovules and seeds suffering an average reduction of 62% (compared to heads without weevil). Kaolin clay did not reduce pollinator visits, visit length or visitor richness. Oviposition was reduced in heads treated with kaolin clay vs. water, and clay-treated heads were less likely to have oviposition at all. Clay-treated heads had 24% more filled seeds, but this treatment did not appear to significantly affect the number of damaged/chewed seeds. I recommend that managers apply kaolin clay to reproductive Pitcher's thistle plants from mid-June through the first week of July, on heads not yet in flower, every 3-7 days to prevent reduction of seed output by individual heads.

2.1 INTRODUCTION

Biological control is the use of a natural predator to control pests in introduced areas. Insects have been used extensively as biological control agents for invasive plants since the late 18th century (van Willgren et al., 2013). Since then, the use of biological control agents has become nationally and internationally regulated, requiring risk assessment for impacts on non-target plant species impacts before release (McEvoy, 1996). Despite these tests, several insects used for biological control have had significant negative impacts on native flora. The cactus moth, *Cactoblastis cactorum* Berg (Pyralidae, Lepidoptera), was originally released in 1957 in the Caribbean to control invasive *Opuntia* (Cactaceae) species (Simmonds and Bennett, 1966). The cactus moth spread to the Southeastern United States in 1989 and currently threatens many native cactus species (Pemberton, 2000).

Another example of non-target impacts from biological control is from the flower head weevil *Rhinocyllus conicus* Frölich (Curculionidae, Coleoptera) first released in the North America in the 1960s to control invasive European thistles (Kok and Surles, 1975). This weevil expanded its host range and has been damaging native thistle species of the western US such as *Cirsium centaurea* (Rydb.) K. Schum., *C. eatonii* (A. Gray) B.L. Rob. and *C. calcareum* (M.E. Jones) Wooton & Standl. (Asteraceae; Louda et al., 1997). Another weevil species, *Larinus planus* Fabricius (Curculionidae, Coleoptera), also has been used to control weedy thistles with documented non-target impacts on Tracy's thistle, *C. undulatum* (Nutt.) Spreng. var. *tracyi* (Rydb.) S.L. Welsh (Louda and O'Brien, 2002) and Pitcher's thistle, *Cirsium pitcheri* (Torr. ex Eat.) Torr. & A. Gray, a threatened Great Lakes dune endemic (Havens et al., 2012).

2.1.1 *Larinus planus* non-target impacts on *Cirsium pitcheri*

Larnius planus or the seed head weevil, first appeared adventively in North American in 1968. It was not intentionally imported, but later was actively distributed as a biocontrol agent (Louda and O'Brien, 2002). The weevil was tested in the 1960s as biological control for European thistles that had invaded North America. The weevil did feed on the non-native thistles, but no North American native thistles were used to test for feeding on other plant species, i.e., non-target impacts (Zwölfer, 1964; Louda and O'Brien, 2002). Further tests were performed by McClay (1990) using non-native target species such as *Cirsium arvense* (L.) Scop. and native non-target *Cirsium* species. According to Louda and O'Brien (2002), the tests were insufficient to detect possible non-target impacts because they had low sample sizes and failed to properly quantify the differences between target and non-target impacts on *Cirsium* species.

Cirsium pitcheri is an herbaceous monocarpic perennial, native to the cobble shorelines and natural sand dunes of the western Great Lakes of the USA and Canada (Lakes Superior, Michigan, Huron) (Voss, 1972). Individuals grow for approximately 4-8 years in a vegetative rosette before bolting and setting on average 1-35 floral heads between June-August, then dying after this single flowering event (termed monocarpic). *C. pitcheri* does not reproduce vegetatively and relies solely on seed set for reproduction (Keddy and Keddy, 1984; Hamzé and Jolls, 2000).

The life cycle of *L. planus* overlaps with the reproductive phase of Pitcher's thistle. The adult weevil emerges from the leaf litter in early summer and mates soon thereafter (Zwölfer, 1964). The female weevil then finds a thistle inflorescence (head) and chews an oviposition hole through the outer bracts (phyllaries). She lays an egg in the hole and packs it in with frass and chewed vegetative matter. The egg develops into a larva inside the head. Larvae eat the developing flower ovules or seeds before they pupate in the head (Volovnik, 1996; Fig. 1). The adult weevil emerges from the thistle head in late summer to early fall to find a place to overwinter in the leaf litter (Zwölfer, 1964; Havens et al., 2015).

Larinus planus was first observed on *C. pitcheri* in Dorr County, WI in 2007. Impact on individual *C. pitcheri* heads was first estimated as 50-100% seed loss (Havens et al., 2012). *L. planus* has since been found in Pitcher's thistle populations in Wisconsin, Indiana, Illinois, and Michigan. Populations of *C. pitcheri* plants in northern lower Michigan showed a range of *L. planus* damage rates of 0-16.7% (flowering plants with evidence of oviposition; Havens et al., 2014). Models based on empirically-determined population growth rates show that *L. planus* seed predation can significantly reduce time to extinction of Pitcher's thistle to just a couple of decades (Havens et al., 2012).

One potential control of *Larinus planus* is the use of Surround® WP, a crop protectant largely made up of kaolin clay (Tessenderlo Kerley Inc. 2008a, b). Kaolin clay is used to reduce sunburn and prevent insect damage on crops. According to the product manufacturers, Surround® WP deters insects by repelling, impeding movement, grasping or egg laying and also reduces feeding. Kaolin clay can also prevent host recognition, alter behavior and induce paralysis and mortality (Tessenderlo Kerley Inc., 2008c). Unlike many traditional insecticides, kaolin clay is certified for use on organic farms (Lapointe et al. 2006; Delate et al. 2008; Silva and Ramalho, 2013) and is safe to use near sources of fresh water without potential drift that might negatively affect aquatic systems (OMRI, 2013; Washington State Department of Agriculture, 2014). The composition of Surround® WP (95% kaolin clay and 5% inert materials) makes it ideal for use near sensitive freshwater systems (Tessenderlo Kerley Inc., 2008b), such as the sand dunes of the Great Lakes. Common insecticides and herbicides have been shown to reduce species richness of aquatic animals such as water fleas, insect predators and amphibians in

Fig. 1. *Larinus planus* behavior on and damage to *Cirsium pitcheri*. A) *L. planus* male (top) and female (bottom) mating on a *C. pitcheri* head. B) Female *L. planus* chewing a hole through a *C. pitcheri* head phyllaries before laying an egg; the head has kaolin clay residue on the outer phyllaries, and a *L. planus* oviposition hole (arrow) next to the tunneling weevil. C) An aborted *C. pitcheri* head. D) The same head in panel C, dissected in the field to show a *L. planus* pupa (left) and ovule damage (right).

aquatic systems (Relyea, 2005). Commonly used insecticides have a negative effect on honey bee, bumble bee and solitary bee foraging behavior and learning/memory ability (Blacquière et al., 2012). Many of the known insect pollinators of *C. pitcheri* are bee species (Keddy and Keddy, 1984; Loveless, 1984; Basket et al., 2011), and products used to control *L. planus* weevils should not negatively impact these important visitors.

2.1.2 Study Questions

Much is still unknown about this antagonistic interaction between *L. planus* and *C. pitcheri*. I sought to answer basic questions about the impact and control of this seed head weevil on Pitcher's thistle that would be useful to land managers: 1) What is the timing or phenology of oviposition? 2) What factors influence oviposition on heads? 3) What are the impacts of oviposition? 4) Does kaolin clay negatively impact *C. pitcheri* insect visitors? 5) Does kaolin clay prevent oviposition and seed set damage?

2.2 MATERIALS AND METHODS

2.2.1 Impacts and Phenology

Early in the growing season (11-19 June 2015) plants with impacts or weevil(s) present were selected for intensive monitoring at three sites in Emmet County, Michigan (Fig. 2). I monitored 19 plants at Sturgeon Bay, Wilderness State Park (SB), 15 plants at Petoskey State Park (PSP) and 10 plants at Cross Village Township Park (CV). Floral stage (0: not flowering through 6: developing/setting seed, stages from Warneke, 2015), head diameter (mm) and number of oviposition holes (Fig. 1C) were recorded for each head on each of the selected plants. Plants were revisited every 3-7 days. New heads produced after the initial survey were added to the monitoring and re-measured in subsequent visits. If heads aborted before flowering, they were dissected in the field to determine weevil presence or absence. Impact of weevils on seed set was determined by harvest of heads as they matured, followed by dissection in the laboratory. Head contents were sorted into three categories: 1) unfilled ovules or flat seeds, 2) filled seeds and 3) ovules/seeds damaged by weevils (chewed). Ovules or seeds were often totally consumed; in these cases, the desiccated corolla attached to part of an ovule or chewed seed was used as an indicator of a potential seed. Characteristic damage by or presence of weevil was also noted for each head. Filled seeds were returned to the field and scattered around the maternal parent plant

Fig. 2. A map of the populations of Pitcher's thistle in Emmet County, MI used for these studies: Sturgeon Bay (SB) in Wilderness State Park, Petoskey State Park (PSP) and Cross Village Township Park (CV). Inset map shows the relative location in the lower peninsula of Michigan, USA.

before 19 August. Heads that had dispersed their seeds, been browsed by animals or that suffered plant/stem/branch mortality before maturity were not included in the analysis.

2.2.2 Kaolin Clay as Potential Control

In summer 2014, I tested the impact of kaolin clay on insect visits to Pitcher's thistle flower heads. From 11-19 July, I selected 90 pairs of reproductive Pitcher's thistle plants with the same number of open heads, no more than 2 m apart at SB. One plant in each pair received aqueous solutions of approximately 6% (minimum manufacturer recommendation, 1 cup clay:1 gallon water) or approximately 12% (maximum manufacturer recommendation, 2 cups clay:1 gallon water) clay-aqueous solution (kaolin clay:water, v:v). I painted the clay on the peduncle and phyllaries of Pitcher's thistle flowering heads and allowed the solutions to dry. The remaining plant in each pair received nothing as a control. I then observed the pairs of plants simultaneously for 10 min intervals as soon as the clay was dry (roughly 15 min), noting the insect species visiting, number of visits and length of visit (sec). If the visitor moved between different flower heads within the same plant in under 5 sec, it was recorded as the same visit. Unknown insects were collected and identified in the lab.

In summer 2015, I tested the effectiveness of kaolin clay in preventing weevil impacts. From 11-21 June, I selected 18 reproductive *C. pitcheri* plants at PSP that had a weevil present. On each plant, 1-8 pairs of heads unimpacted by *Larinus planus* were selected based on the likelihood of flowering and similarity of diameters at time of selection (within 2-3 mm). Paired heads randomly received either 6% clay-aqueous solution (kaolin clay:water, v:v) or a water control, applied with a paintbrush to the phyllaries and peduncle. Plants were revisited every 3-7 days from 11 July to 26 July for reapplication of kaolin clay or water and a count of oviposition holes. Reapplication was necessary since the kaolin clay residue film washes off after rain events and in high wind conditions. Between 26 July and 14 August, heads were collected for seed counts in the laboratory. The same methods were used for seed set analysis as in the "Impacts and Phenology" descriptive work (above).

2.2.3 Statistical Analysis

I used a generalized linear mixed binary logistic model to analyze the use of fixed effect variables to predict oviposition on Pitcher's thistle heads. The binary outcome of oviposition (1 = yes and 0 = no) was modeled as a function of x, y and z. Date of oviposition (Date), x, was converted to an integer reflecting the number of days since adult weevils were first observed on Pitcher's thistle plants (11 June 2015). Size of the head at oviposition (Size), *y,* was recorded to the nearest 0.5 mm. Head development (Floral Stage), *z*, reflects whether the head was in bud (0) or in bloom (1) at oviposition. I also incorporated a two-way fixed effect of Date and Size into the model. If the heads did not receive oviposition I used the size and floral stage measurements from the date of peak oviposition at each of the three sites: SB, 7 July; PSP, 30 June; CV, 27 June (Fig. 3). I used a mixed model as there were several heads on each of the 44 thistle plants from the three sites (SB: 19 plants, 548 heads; PSP: 15 plants, 285 heads, CV: 10 plants, 136 heads; Total: 44 plants, 969 heads). This approach was a means of incorporating a cluster structure, which created an intra-plant correlation among multiple heads on a given plant. The model used a variance component structure that incorporated a random effect for plant. The random component was assumed normally distributed with a mean of zero and a variance of σ^2_p. The random component created the structure for intra-plant correlation among the multiple heads.

$$\text{Logit(oviposition)}_{ij} = a + \beta_1 x + \beta_2 y + \beta_3 z + \beta_4 xy + e_{ij}$$

$$i^{th} \text{ plant, } j^{th} \text{ head on plant i, } i = 1,\ldots n_p,\ j = 1,\ldots,r_i.$$

The intercept *a*, reflects natural log odds when all covariates *x*, *y*, *z* are zero. The coefficients β_1, β_2, β_3 and β_4 are the increases in the log odds of oviposition for a unit increase in the respective covariate. The e_{ij} is a random error term associated with the j^{th} head on the i^{th} plant. This error term is assumed normally distributed with a mean of 0, and variance σ^2_p. The model was constructed using SPSS Statistics v. 22.0 (IBM Corp., 2013).

I used paired t-tests to analyze mean number of visits, length of visit (sec) and species richness between the kaolin clay treatment groups and the respective control plants to determine whether kaolin clay has the potential to negatively impact the pollinator fauna. I compared mean counts of each of the seed categories with Student t-tests (assuming equal or unequal variance as appropriate) to quantify the effect on weevil presence on seed output per *C. pitcheri* head. I also performed a Pearson's Chi-square test on counts of heads to compare whether oviposition was present or absence was contingent upon treatment (kaolin clay or water treatment) of individual heads. Means ± standard error are reported throughout.

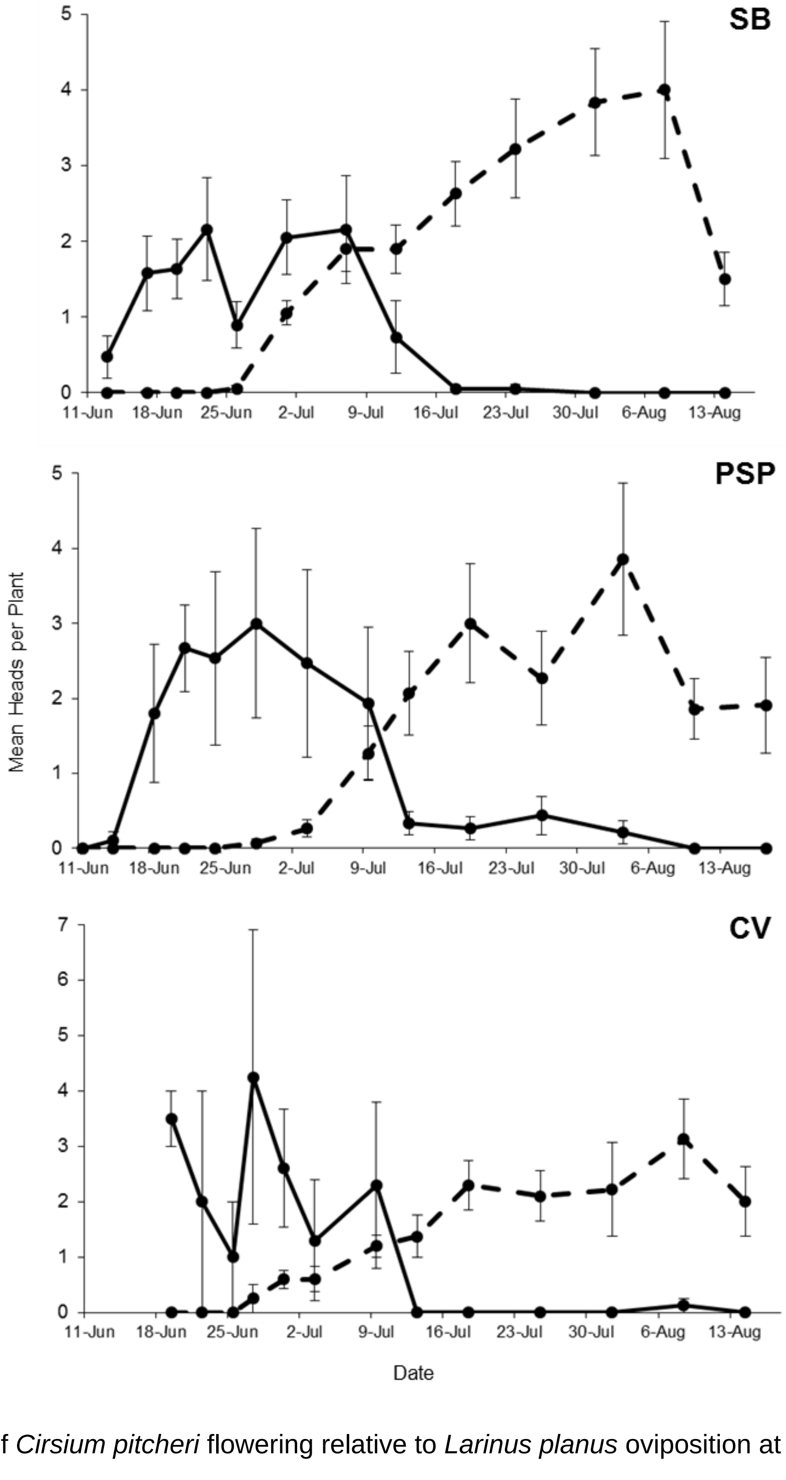

Fig. 3. Phenology of *Cirsium pitcheri* flowering relative to *Larinus planus* oviposition at three sites in 2015. Mean (± SE) number of heads per plant that received first oviposition (solid line) or were in bloom (dashed line) 11 June-17 August 2015. Panels describe sites: SB = Sturgeon Bay, 19 plants; PSP = Petoskey State Park, 15 plants; CV = Cross Village Township Park, 10 plants.

2.3 RESULTS

2.3.1 Impacts and Phenology

Weevil impacts on Pitcher's thistle were first noted on 13 June 2015 at SB, 11 June at PSP and 19 June at CV with peak oviposition at 7 July, 28 June and 27 June respectively. By mid-July, virtually all oviposition had ceased (Fig. 3). The majority of oviposition occurred before or early during *Cirsium pitcheri* flowering (Fig. 3). I revisited 1,695 heads on 44 plants at the three sites; 540 (32%) heads had oviposition by the end of sampling in mid-August. Head size at first oviposition averaged 12.4 ± 0.2 mm ($\bar{X}$ ± SE) in diameter (Table 1). The majority of heads that received oviposition were less than 15 mm in diameter (Fig. 4). I dissected a total of 452 heads in the field and lab; of those heads, 369 (82%) had oviposition. Of dissected heads that had oviposition, 164 heads (44%) showed evidence of weevil damage to ovules or seeds and 205 (56%) showed no damage (Table 2).

I compared ovule and seed fate in heads damaged by weevils to those not damaged. Significant differences were found between damaged and undamaged heads for means of all seed categories: total potential seed ($t = 2.213$, df = 166, $p = 0.028$), unfilled/flat seed ($t = 2.057$, df = 148, $p = 0.041$), filled seed counts ($t = 8.046$, df = 179, $p < 0.001$) and chewed seed ($t = 12.182$, df = 77, $p < 0.001$; Fig. 5). The mean number of filled seeds in weevil-damaged heads was 24.3 ± 3.87 compared to 64.2 ± 3.11 in undamaged heads (Fig. 5). Thus weevil oviposition and development reduced viable seed set by 62%.

The generalized linear mixed binary logistic model predicted head oviposition with 84% overall accuracy (Table 3). The area under the receiver operating characteristic curve (AUC) was 0.896 ± 0.100. Date (odds ratio 54.100, $p > 0.001$) and Size (odds ratio 6.535, $p = 0.011$), were significant predictors of oviposition. Floral Stage (odds ratio 3.679, $p = 0.055$) and the two-way effect of Date and Size (odds ratio 0.071, $p = 0.790$) were not significant predictors of weevil attack of heads (Table 4). Size of heads may have been a significant predictor of oviposition because there was a narrow size range of heads on which weevils oviposited on (Table 1).

2.3.2 Kaolin Clay Experiments

We found no significant differences in mean number of insect visits, mean duration of visit (sec) and species richness values during 10 min observations between kaolin clay-treated and control plants (Table 5). Kaolin clay-treated heads had significantly fewer mean oviposition holes compared to water-

Table 1. Sizes of *Cirsium pitcheri* heads (mm) at the time of receiving first oviposition from *Larinus planus*, at each study site.

Site	Head Size (mm) $\bar{X}$ ± SE (*n*)
Sturgeon Bay	12.3 ± 0.28 (248)
Petoskey State Park	11.5 ± 0.30 (218)
Cross Village	14.3 ± 0.58 (93)
All Sites	12.5 ± 0.20 (559)

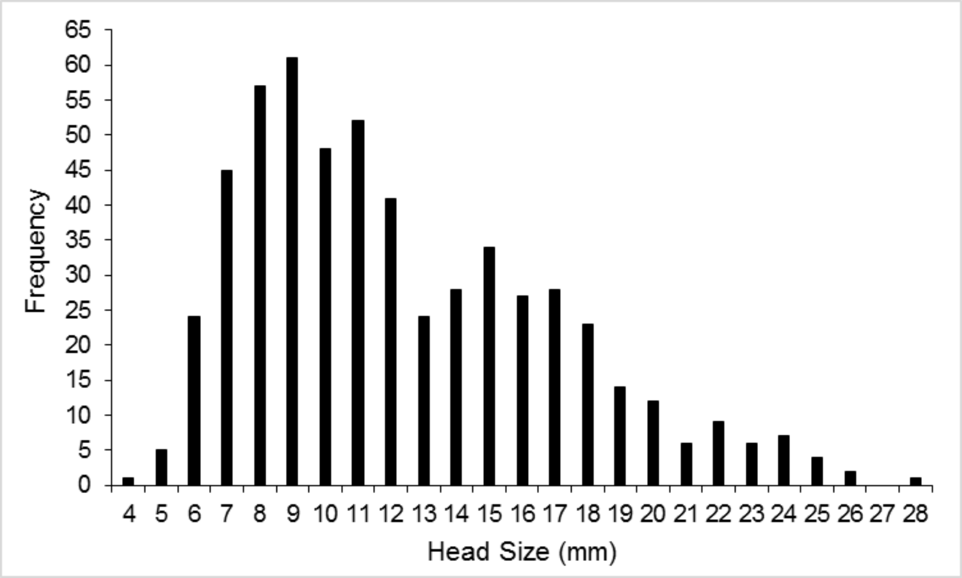

Fig. 4. Frequency of *Cirsium pitcheri* head sizes recorded after receiving first oviposition from *Larinus planus* in summer 2015 from three populations in Emmet County, MI (*n* = 540).

Table 2. Summary of weevil impacts on dissected heads of *Cirsium pitcheri* surveyed in 2015. Total Heads Dissected represents the number of heads that were opened either in the field or in the laboratory to check for weevil presence. Under the Dissected Heads with Oviposition category, the Total (%) represents the count of dissected heads with oviposition and the percentage using Total Heads Dissected as the quotient. Weevil Absent (%) represents the mortality of the weevil egg before damaging *C. pitcheri* ovules or seeds. Weevil Present (%) represents the likelihood of a weevil egg developing into a larvae and damaging *C. pitcheri* ovules or seeds.

Location	Total Heads Dissected	Dissected Heads with Oviposition		
		Total (%)	Weevil Absent (%)	Weevil Present (%)
SB	215	162 (75.4%)	89 (54.9%)	73 (45.1%)
PSP	178	152 (85.4%)	76 (50.0%)	76 (50.0%)
CV	59	55 (93.2%)	40 (72.7%)	15 (27.3%)
All Sites	452	369 (81.6%)	205 (55.6%)	164 (44.4%)

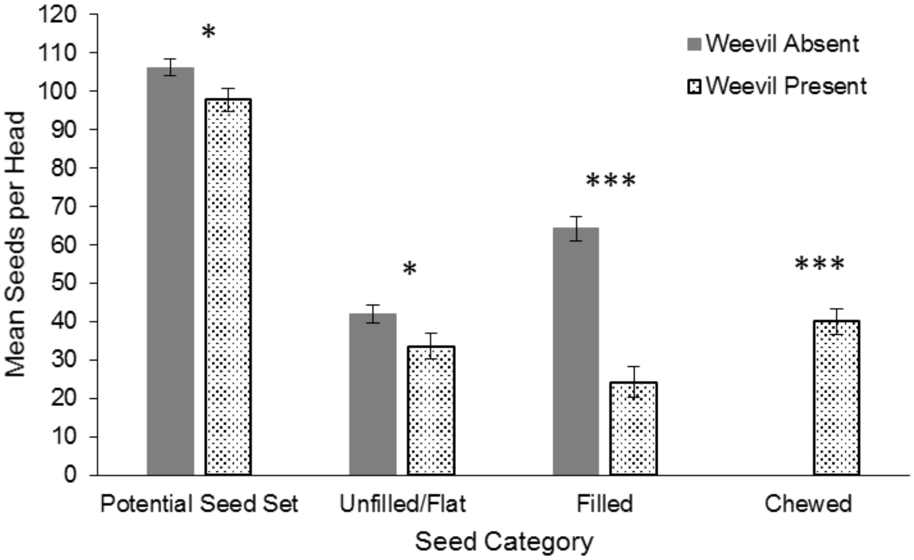

Fig. 5. Mean (± SE) seed set per category for *Cirsium pitcheri* heads with no evidence of *Larinus planus* damage (Weevil Absent; *n* = 194) and evidence of *L. planus* damage (Weevil Present; *n* = 78). Potential Seed Set category is the sum of Unfilled/Flat, Filled and Chewed categories. The Weevil Absent Chewed seed category reflects zero counts for all heads included in the analysis. Student t-tests assuming unequal variance showed significant differences between weevil present and weevil absent for means of all seed categories. (*) $p<0.05$, (**) $p<0.01$, (***) $p<0.001$.

Table 3. Classification performance of the generalized linear mixed binary logistic model predicting oviposition from the seed head weevil on Pitcher's thistle heads. The model used fixed effect predictors of date of oviposition, size of head (mm), head floral stage and a two-way effect of date and size. Raw values and percentages of observed row totals are displayed for each cell representing 969 total heads in the model predicting oviposition with 83.9% accuracy.

Observed	Predicted		Total
	No Oviposition	Yes Oviposition	
No Oviposition	530 (92.7%)	42 (7.3%)	572
Yes Oviposition	114 (28.7%)	283 (71.3%)	397

Table 4. Coefficients of the fixed effect variables of the generalized linear mixed binary logistic model predicting oviposition.

Model Term	Coefficient β (± SE)	t	p
Intercept	-1.25 (1.512)	-0.827	0.408
Date	0.20 (0.027)	7.355	< 0.001
Size	-0.12 (0.046)	-2.556	0.011
Floral Stage	-1.15 (0.602)	-1.918	0.055
Date x Size	-0.001 (0.002)	-0.267	0.790

Table 5: Number of insect visits, duration of visit (sec) and species richness of the insect visitors to pairs of Pitcher’s thistle plants. The pairs were treated with either 6% kaolin clay:water (v:v) or nothing (control), or 12% kaolin clay or nothing, then simultaneously observed for 10 min. Paired t-tests assuming equal variance were used to compare the two treatment levels and the respective controls.

Treatment	Visits		Duration (sec)		Species Richness	
	$\bar{X}$ ± SE	Paired t-test	$\bar{X}$ ± SE	Paired t-test	$\bar{X}$ ± SE	Paired t-test
6% Clay	2.4 ± 0.29	t_{44} = 0.231, p = 0.817	73.9 ± 14.52	t_{44} = 0.892, p = 0.408	1.7 ± 0.19	t_{44} = 0.822, p = 0.415
Control	2.3 ± 0.26		101.6 ± 18.75		1.5 ± 0.15	
12% Clay	2.6 ± 0.39	t_{44} = 0.800, p = 0.428	99.7 ± 23.032	t_{44} = 0.138, p = 0.891	1.6 ± 0.17	t_{44} = 0.127, p = 0.900
Control	2.3 ± 0.30		88.3 ± 18.36		1.6 ± 0.18	

treated heads ($t = 5.136$, $df = 55$, $p < 0.001$; Fig. 6). There were significantly more filled seeds in kaolin clay-treated compared with water-treated heads ($t = 2.036$, $df = 77$, $p = 0.023$); however, there were no significant differences in the number of chewed ($t = 0.85$, $df = 75$, $p = 0.427$) or unfilled/flat seeds ($t = 0.783$, $df = 81$, $p = 0.436$) between clay- and water-treated heads (Fig. 7). Whether or not oviposition occurred was dependent on treatment (kaolin clay vs. water, $X^2 = 7.092$, $df = 1$, $p = 0.008$; Table 6).

2.4 DISCUSSION

The phenology of *L. planus* oviposition exhibits strong overlap with the early period of *C. pitcheri* reproduction when heads are largely in bud. Oviposition took place from mid-June to early July on heads of a narrow size range (12-14 mm in diameter) before they flowered (Table 1, Fig. 3). It appears that weevils prefer heads of a smaller size range; the majority of oviposition occurs on heads ≤ 15 mm in diameter (Fig. 4). This size range reflects the smaller heads available early in the season. The generalized linear mixed binary logistic model showed that the seasonal timing (Date) and the size of Pitcher's thistle heads were significant predictors of oviposition (Table 4). I expected that Floral Stage would be a significant fixed effect, since weevils are largely selecting heads in bud for oviposition. However, the lack of significant effect of Floral Stage was likely due to so few heads in flower at first oviposition (only 18 out of 969 heads included in the model showed any open florets). Van Hezewijk and Bourchier (2012) also found mean diameter of heads of the target weed diffuse knapweed (*Centaurea diffusa* Lam., Asteraceae) was a highly significant predictor of the proportion of heads attacked by *Larinus minutus* Gyllenhal; Curculionidae: Coleoptera) in a generalized linear model. This stage- and size-selectivity by *Larinus planus* suggests that some heads or even entire plants may escape weevil damage based on flowering time relative to weevil abundance, mating and oviposition. Reproductive Pitcher's thistle plants add heads throughout the growing season (Keddy and Keddy, 1984). Heads that are added to the plant after the peak oviposition period may escape weevil damage and have the potential to produce viable seeds. Of the 44 plants with early weevil damage selected at the three sites, only 32% of the 1,695 heads had oviposition. Even once a plant is found by a weevil, heads may escape depredation. In estimates of *Larinus planus* non-target damage on the native thistle *Cirsium undulatum* in Colorado, approximately 35% of all heads surveyed in two populations showed unambiguous external feeding by *L. planus* on the seed head characteristic of oviposition (Louda and O'Brien, 2002). I found that 56% of

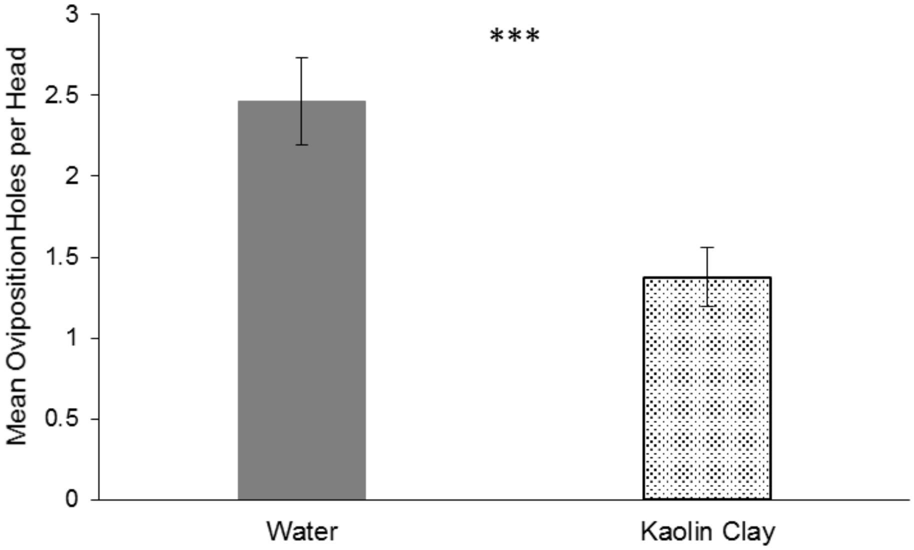

Fig. 6. Mean (± SE) number of oviposition holes per head for 56 pairs of water or kaolin clay-treated Pitcher's thistle heads on 18 plants at Petoskey State Park, Emmet County, MI. A paired t-test indicated significantly more oviposition holes on water-treated heads than kaolin clay-treated heads. (***) $p < 0.001$.

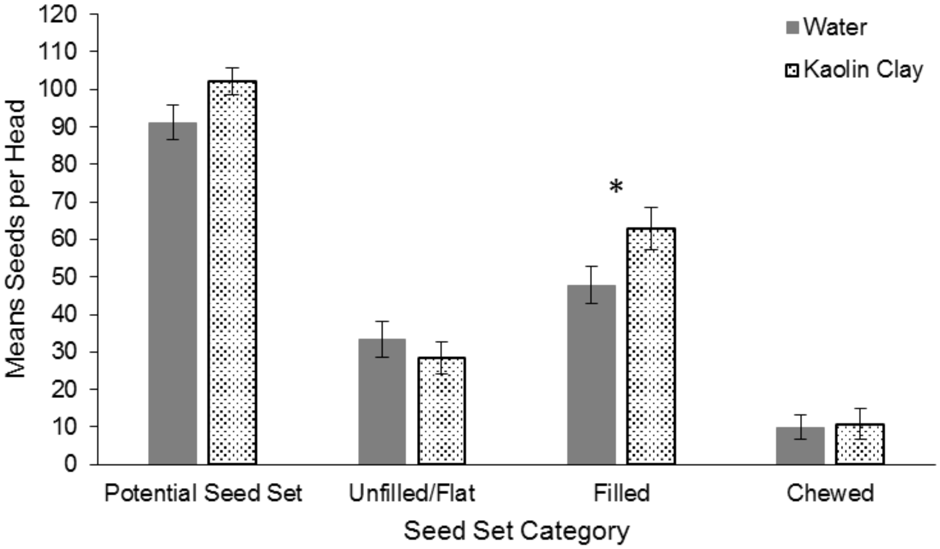

Fig. 7. Mean (± SE) numbers of seeds per category of *Cirsium pitcheri* heads treated with water (*n* = 45) and kaolin clay (*n* = 38). The Potential Seed Set category is the sum of Unfilled/Flat, Filled and Chewed categories. There were significantly more filled seeds in kaolin clay treated heads than water treated heads. (*) $p < 0.05$.

Table 6. Contingency table for the number of heads that received oviposition associated with the kaolin clay or water treatment groups. Percentages reflect counts for the treatment row totals. A Pearson's Chi-square test showed non-independence between the treatment groups and oviposition presence or absence.

Treatment	Oviposition Absent (%)	Oviposition Present (%)	Total
Kaolin Clay	16 (28.6%)	40 (71.4%)	56
Water	5 (8.9 %)	51 (91.1%)	56
Total	21 (18.8%)	91 (81.2%)	112

dissected *C. pitcheri* heads with evidence of oviposition did not have a weevil or characteristic damage to ovules or seeds (Table 2, Fig. 1). This suggests that more than half of the eggs once in a thistle head do not survive to the larval and pupal stages to damage ovules or seeds. Similarly, the related biocontrol *Rhinocyllus conicus* reduced the seed set *Cirsium ownbeyi* S.L. Welsh in Wyoming by 95% in infected heads, but only half the heads on any reproductive plant were infected (Deprenger-Levin et al, 2010). It is also possible that *L. planus* adults fed on the *C. pitcheri* heads but did not oviposit.

Larinus planus weevils do have a significant negative impact on seed production by heads of *C. pitcheri*. On average, weevil presence was associated with chewed seeds or ovules and a reduction in the number of filled seeds per head by 62%; it is these filled seeds that are most likely to be viable. *L. planus* reduced the number of viable seeds from 44.5 ± 2.98 in undamaged heads to 1.4 ± 0.40 in damaged heads of *Cirsium undulatum* in Colorado (Louda and O'Brien, 2002). I also found significant differences between potential seed set category and the unfilled/flat seeds in heads with weevil present vs. absent. We may not necessarily expect a difference in the potential seed set (total number of ovules per head) for inflorescences with or without a seed predator. My method may have underestimated potential seed set in heads with weevil present. It was difficult to count the number of chewed seeds using the desiccated floret as a proxy for the destroyed ovule or seed. Alternatively, selection of thistle inflorescence buds by ovipositing females may destroy the developing ovules and florets thus reduced potential seed set.

The non-target effects from *L planus* on *C. pitcheri* have population-level implications, although their magnitude may be as yet unknown. My work did not directly account for the population-level effect of weevil damage associated with its reduction of seed output. Other work, however, has shown reductions in population viability of Pitcher's thistle from weevil seed predation (Havens et al., 2012). Pitcher's thistle has no means of vegetative reproduction and therefore relies solely on successful seed set and germination for population persistence and survival (Loveless, 1984; Hamzé and Jolls, 2000). Havens et al. (2012) used population viability analysis to estimate *C. pitcheri* time to extinction with *L. planus* damage estimated with 50% reduction of fecundity (reproductive plants to seedlings the subsequent year). This estimate was based on seed set estimates from heads collected from Whitefish Dunes, WI, and did not account for weevil mortality inside the heads that left seeds undamaged. My counts show that on plants impacted by *L. planus*, 32% of heads receive oviposition; of those initially impacted, only 44% have a weevil surviving to damage seeds. Thus, approximately 14% of heads on plants selected by weevils will be impacted. Only then is seed set reduced on average by 62%. This estimate of 50-100% reduction in fecundity may be too high for future iterations of population models for the sites in northern lower Michigan. This estimate may be better suited for Whitefish Dunes in Wisconsin that may have a higher frequency of oviposition and perhaps higher survival of weevil larvae (Havens et al., 2015).

We need to better understand which Pitcher's thistle plants are at risk, perhaps related to microsite abiotic factors (Volkl et al., 1993) and density of flowering plants (e.g., resource abundance patterns of Stephens and Myers, 2012). I selected plants for intensive monitoring based on those already selected by the weevils that first emerged from the leaf litter in mid-June. These were the first plants in the populations to show signs of *L. planus* presence or damage, which may have biased my selection. The necessary next step is to compare plants that were not damaged by weevils by those that were. Other studies of non-target impacts from these two biocontrol weevil species on other native *Cirsium* species in North America used methods slightly different from mine to select plants to quantify weevil impact across whole populations of thistles. Louda and O'Brien (2002) randomly chose plants from the populations of *C. undulatum.* DePrenger-Levin et al. (2010) randomly collected three reproductive *C. ownbeyi* plants from their demography plots. Nonetheless, these other studies report extent of damage to congeners comparable to what I observed for Pitcher's thistle.

Phenology, or the timing of biological events, within and among species, is important in quantifying the severity of non-target impacts of a biological control agent. Originally, the biocontrol weevil *Rhinocyllus conicus* was intended to only impact the invasive musk thistle *Carduus nutans* L., but it has had non-target impacts on many species of native thistles (Kok and Surles, 1975; Louda et al; 1997; Pemberton, 2000). In Nebraska, this weevil was documented to oviposit on the native wavyleaf thistle (*Cirsium undulatum* var. *tracyi*) early in the season when flower heads were abundant. *R. conicus* then oviposited more frequently on the native Platte thistle (*Cirsium canescens*) when its flower heads became more abundant later in the growing season. These two native thistles mediated the effects of the invasive weevil by presenting resources at different times in the growing season (Russell and Louda, 2005). In the Pitcher's thistle populations surveyed in northern lower Michigan, I found no alternate hosts (*Cirsium* or

Carduus of tribe Cardueae of the Asteraceae family) in the dune ecosystems I studied to potentially mediate the impacts of *L. planus*; other systems can have higher abundances of related invasives, e.g., *Cirsium arvense, C. vulgare, C. nutans*. In both cases, however, the reproductive phases of these Nebraska and Michigan thistles co-occur with the mating and oviposition of the biocontrol weevils. This suggests that an understanding of timing of the availability as well as choice of alternative hosts of biological controls is important for assessment of potential non-target impacts.

Prevention of *L. planus* damage is vitally important for the conservation and population persistence of Pitcher's thistle. Kaolin clay is an ideal control method since it is certified organic and is safe to use near sources of freshwater such as the Great Lakes (OMRI, 2013; Washington State Department of Agriculture, 2014). Additionally, kaolin clay does not change the visitation or behavior of insect-flower visitors of *C. pitcheri*. The 2015 experiments showed that kaolin clay does reduce the number of ovipositions per head, on average and increased mean filled seeds per head by 24% compared to the water-treated heads (Fig. 6, 7). The effectiveness of kaolin clay results from the prevention of oviposition. Clay-treated heads were more likely to escape oviposition than water-treated heads (Table 6).

Kaolin clay has been shown to be limited in its effectiveness in preventing insect damage on agricultural plants as well as on Pitcher's thistle. It may serve as one approach to controlling the impact of *Larinus planus*. The use of kaolin clay reduced oviposition of a root weevil on citrus crops but did not prohibit all damage (Lapointe et al., 2006). Similarly, kaolin clay significantly reduced oviposition damage by boll weevil to cotton compared to the control treatment, but endosulphan insecticides were still more effective (Silva and Ramalho, 2013). The manufacturers do not claim this kaolin clay product will prevent all insect damage, but rather reduce the impacts from insects (Tessenderlo Kerley Inc., 2008c). Kaolin clay also has been used to reduce drought stress in olive trees and significantly reduced leaf temperature compared to the control (Denaxa et al., 2012). Although I did not measure temperature of *C. pitcheri* heads, this unintended effect of reducing plant tissue temperature may also have contributed to production of more filled seeds in clay-treated heads.

Understanding the selectivity, phenology and extent of impacts of this weevil on Pitcher's thistle is important for the conservation of this federally threatened dune endemic. Populations of *C. pitcheri* already suffer from many other threats including coastal habitat degradation (Rankin and Crispin, 1994), low genetic diversity (Gauthier et al., 2010; Fant et al., 2013), invasive species encroachment (Baskett et al. 2011; Emery et al., 2013), low germination (Hamzé and Jolls, 2000), climate change induced habitat narrowing (Vitt et al. 2010), and herbivory by native birds, mammals and other insects (Phillips and Maun, 1996; Stanforth et al., 1997; Bevill et al., 1999; Rowland and Maun, 2001; Havens et al. 2012). This plant may also be an important member of the dune ecosystem as a floral resource for the insect fauna, particularly given its flowering period (late June through July) relative to other dune plant species. Loss of this species, due to weevil impacts or other threats, could have the potential to significantly impact the dune ecosystem plant-pollinator network in a negative way.

2.4.1 Conclusions and Management Recommendations

Seed set of Pitcher’s thistle is reduced by 62% in heads that first receive oviposition early after weevils emerge, provided eggs then mature to larval or pupal stages. Oviposition primarily takes place mid-June to early July on heads that are not yet flowering. This time window is critical for implementing management to prevent weevil damage. The short time window of oviposition, however, may make it easier for managers to control *L. planus* negative impacts on *C. pitcheri* since the impacts do not occur over many weeks or months. Kaolin clay-treated head show reduces oviposition and are less likely to have oviposition at all. These heads produced significantly more seeds per head compared to the water control. Land managers should consider applying kaolin clay to reproductive Pitcher’s thistle plants mid-June to early July on any heads not yet in bloom, every 3-7 days. Protection and enhancement of seed set and seedling success are vital for persistence of this distinctive endemic of the Great Lakes shorelines.

CHAPTER 3: PITCHER'S THISTLE IS AN IMPORTANT FLORAL RESOURCE IN THE GREAT LAKES DUNE PLANT-INSECT VISITOR NETWORK

ABSTRACT

Pitcher's thistle (*Cirsium pitcheri*) is a threatened plant endemic to dunes of the Great Lakes. It flowers late-June to early-August at a time when other floral resources are less abundant or unavailable. I hypothesized that during flowering, *C. pitcheri* is an important species for insect flower-visitors in the dune ecosystem. To test this, I performed 10 min insect visitor observations on all insect pollinated plants in 10 m by 10 m plots at Sturgeon Bay of Wilderness State Park, Emmet County, MI, 26 June-5 August 2015. I recorded plant species, number of open flowers, species of insect visiting and number of visits. I found that Pitcher's thistle received 18.2% (109) of all 600 recorded visits, 37.6% more than the next most visited plant. Pitcher's thistle also received visits from 30.5% (22) of the 59 insect species in the network, twice as many insect species as *Dasiphora fruticosa,* the plant species visited by the next most diverse set of insect. Species-level network analysis metrics showed that Pitcher's thistle was the most generalized (*d'* = 0.368), had the highest species strength (9.525) and greatest weighted betweenness (0.310) and weighted connectance scores (0.029) of any other plant species, demonstrating network topological importance. Pitcher's thistle was as abundant as most other plant species (measured either as numbers of plants or numbers of flowers), but still received significantly more insect visits. I concluded that Pitcher's thistle is an important floral resource in the dune network, potentially at the keystone level. Conservation of *C. pitcheri* and its threatened dune ecosystem should be a priority for land management objectives.

3.1 INTRODUCTION

Network analysis has been used to assess the topological importance, or the level of support in network stability, of individual species within ecological networks (Jordán et al., 2008). A plant-pollinator network is defined as the cumulative interactions between plants and their animal pollinators in an ecosystem. These networks are directed, meaning the interactions are one way; insects and plants interact with one another but insects do not interact directly with other insects and plants do not interact directly with other plants. Plants that attract a wide variety of visitors (several orders of insect or animal) are considered generalists, as opposed to specialists, which attract few visitors (one order of insect or a few specific species) (Waser et al., 1996). Plants are considered generalists not only when considering the number of pollinator species, but also the number of visits of each interaction relative to the rest of the network (Blüthgen et al. 2006; Sahli and Conner, 2006). The extinction of highly connected species can result in a cascade of secondary extinctions of species dependent upon the initial extinct species. The resistance to secondary extinctions provides a measure of network resilience (Memmot et al. 2004).

A keystone species was originally defined as a predator species that holds in check other species that would otherwise dominate the system (Paine, 1969). A more modern definition of keystone is a species whose effect is large and disproportionately so in relation to body size and abundance (Power et al., 1996). This concept can be applied to plant-pollinator interactions by analyzing the number of connections and the strength of those connections to other species (Martín Gonzáles, 2010; Pocock et al., 2011). Network analysis species level metrics such as connectedness, betweenness, the index *d'*—a measure of an individual species generality or specificity, and species strength—a sum of insect dependencies on a particular plant, have been used to identify important potentially keystone plants in plant-pollinator networks (Martín Gonzáles, 2010; Pocock et al., 2011; Robson, 2014 Koski et al. 2015).

Individual plants species, even rare or endemic taxa, can be important members of plant-pollinator networks. One network analysis showed that rare endemic plants of oceanic island networks have higher linkage to pollinators than do non-endemic native plants (Olesen et al., 2002). This study also suggested that robustness in a plant-pollinator network can be partially attributed to the presence of native plant species. A rare endemic clover species of the sand dunes of Manitoba and Saskatchewan, Canada (*Dalea villosa* var. *villosa* [Nutt.] Spreng, Fabaceae) is an important nectar and pollen resource for insects, with significantly higher visitation rates than all the other plant species present (Robson, 2014). Rare dune species in other ecosystems may have the same potential.

Pitcher's thistle (*Cirsium pitcheri* [Torr. ex Eat.] Torr. & A. Gray, Asteraceae) is a federally threatened species, endemic to the western Great Lakes sand dunes and cobble shores. It is an herbaceous perennial, growing for 4-8 years in a vegetative rosette. In its last year of life, the plant bolts and produces an average of 1-35 floral heads per plant before setting seed and then dies (monocarpic). The plant has no means of vegetative reproduction and relies solely on seed set for reproduction (Loveless, 1984; Keddy and Keddy, 1984; Hamzé and Jolls, 2000). Bagged inflorescences (heads or capitula) do produce seed, confirming potential for self-pollination within a head in the absence of an insect visit (autogamy). However, insect transfer of pollen is important for seed set, as true for many Asteraceae. Selfing can occur via pollinator movements within and among heads on the same plant (geitonogamy), but more seeds per head are produced with insect-mediated outcrossing (Loveless, 1984). The reproductive plant is primarily pollinated by members of the genus *Bombus* and the bee family Halictidae, but visits from other solitary bees, butterflies, flies and beetles have also been documented (Keddy and Keddy, 1984; Loveless, 1984; Baskett et al., 2011). This wide variety of insect visitors suggests *C. pitcheri* may be a generalist and could function as an important floral resource in the Great Lakes natural dune ecosystem. Pitcher's thistle flowers from late June to early August at a time during which other floral resources in the dune ecosystem may not be present or as abundant (Voss, 1972; Goodwillie and Jolls, 2013; Fig. 8). Although this plant is threatened and limited in its distribution, I hypothesized that *C. pitcheri* is a valuable floral resource to the insect fauna of the local dune ecosystem during its flowering period.

3.2 METHODS

3.2.1 Field Work

I observed insect visitation on all insect-pollinated flowering plants in a population of Pitcher's thistle in randomly selected plots in Sturgeon Bay of Wilderness State Park, Emmett County, MI. Insect-pollinated taxa were assumed to be those with showy flowers. I based this study at Sturgeon Bay because it has minimal negative impacts from human use of the coastal shoreline, the floral diversity is higher than neighboring sites, and the population of Pitcher's thistle is relatively large. I used ArcGIS 10.1 software (ESRI, 2013) and satellite imagery, to create a 0.255 km^2 polygon around the dune ecosystem along 2,461.5 m (2.46 km) of shoreline. I randomized 60 points in the polygon to represent the center of 10 m by 10 m plots. I performed 10 min observations in warm, low wind weather between 1000 h and 1500 h on all the insect pollinated flowering plants during the *C. pitcheri* flowering season, 26 June-5 August 2015. If there were no flowering plants in a plot, I did not perform any observations. I recorded plant species, number of open flowers, insect species visitors and number of each visit. For plants in the family Asteraceae, the head (inflorescence) was considered one flower. A visit was counted if the insect made contact with floral parts of the plant (i.e., petals, stamens, stigmas). If visitors were not identifiable in

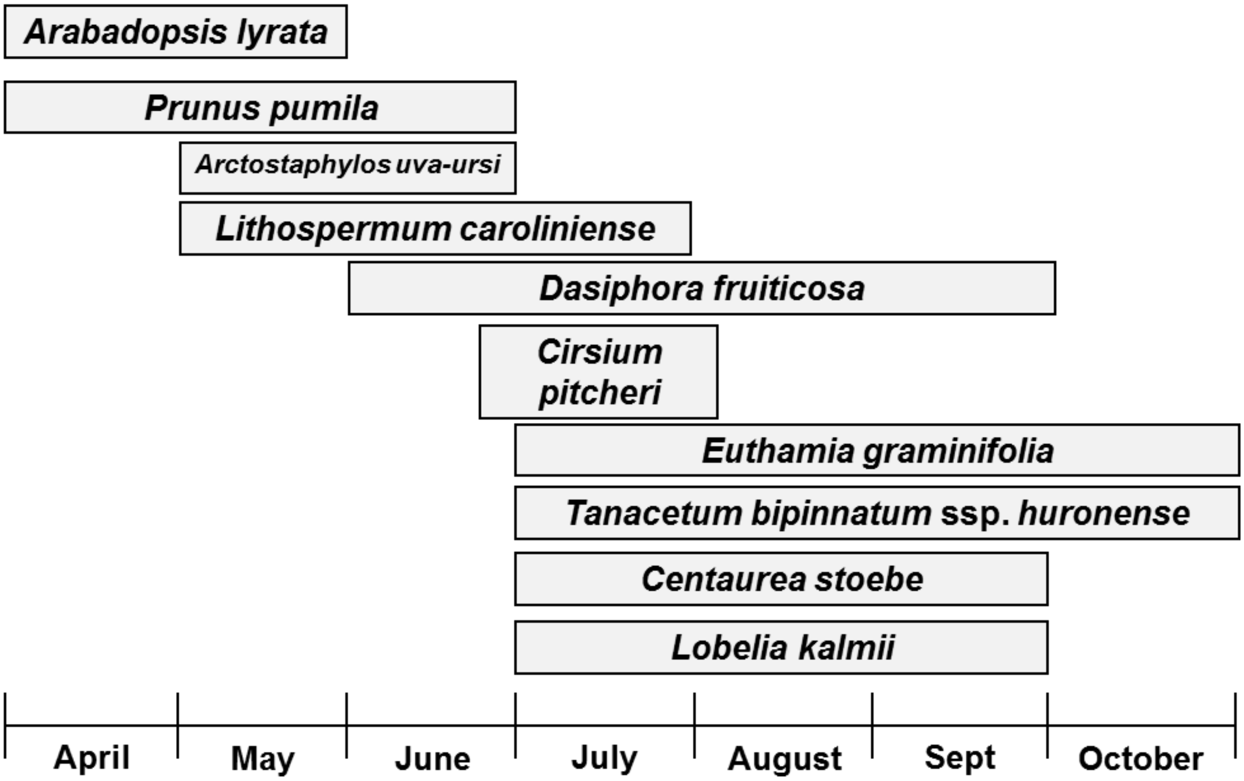

Fig. 8. Phenology of flowering periods of Great Lakes dune insect pollinated plant species. Flowering period data from floras (Voss, 1972; Ownbey and Morley, 1991; Flora of North America Editorial Committee, 1993; Chadde, 2013; Fig. 3).

the field, I collected the insect for later identification. When individual plants of the same species were within 1 m of one another, multiple plants were observed for visits at the same time to reduce the field time spent in a single plot. A total of 14,010 min of observation with 600 insect visits was recorded in 44 plots.

Observations were recorded on 30 plant species but nine species never received insect visits and were not included in the network. I also removed three species of crab spiders (Thomisidae, Aranae) recorded visiting two plant species from the final network. These spiders use flowers as a habitat structure to lure and prey on flower pollinators (Morse, 1979) and are therefore at a higher trophic level than the insects visiting flowers for pollen and nectar resources. We retained the insect predator *Nabicula subcoleoptrata* Kirby (Nabidae; Hemiptera) in the network since it has also been recorded visiting related *Cirsium* genus members for pollen and nectar resources in neighboring Wisconsin (Williams, 2015). Some *Bombus* species are notoriously difficult to identify, particularly on-the-wing; recorded observations of some species were identified as *Bombus* sp. for network analysis. These unknown *Bombus* visitors may have been *B. griseocolis* De Geer or *B. bimaculatus* Cresson (Apidae: Hymenoptera), both of which were not confused with the other two distinctive *Bombus* species (*B. impatiens* Cresson and *B. ternarius* Say) in on-the-wing identification. The plant-insect visitor network consisted of 21 plants species and 59 insect species (Appendix A).

3.2.2 Network Analysis

To assess the relative importance of each plant species, I calculated seven species-level indices using R statistical software package Bipartite (Dorrman et al., 2008; R Core Team, 2013). Normalized degree (ND) is the number of insect species that visited a plant divided by the number of potential insect visitors in the network. Species strength (SS) is a sum of the insect visitor dependencies on the plant species. Betweenness (B) is a measure of centrality for nodes in the network, quantifying the number of times a plant bridges the shortest path between two other nodes, or the species importance as a connector. Closeness (C) similarly describes the proximity of a species to the rest of the network by quantifying the length of each path to the rest of the nodes (Martín Gonzalés et al., 2010). Betweenness and Closeness values report unweighted and weighted (BW and CW) by the number of visits the plant species received in the whole network. The metric *d'* is an index of specialization measured by the number of visitors a plant has, and is derived from information theory (Blüthgen et al., 2006) The index is scaled from 0 to 1, 0 being the most generalized species and 1 being the most specialized. The Betweenness, Closeness, and *d'* species-level indices have been used to theoretically identify topologically important species in other networks (Martín González et al., 2010; Pocock et al. 2011).

3.3 RESULTS

There were six orders of insects in the entire network with 23 Hymenoptera (bees, wasps and ants) representing the largest group. Hymenopterans also performed over half the visits to plants (376 visits of 600 total). The insect species with the most visits was the unknown bumble bee, *Bombus* sp. (Apidae, Hymenoptera; 96 visits) with the ant *Formica argentea* Wheeler, W.M. (Formicidae, Hymenoptera; 91 visits) at a close second (Table 7, Fig. 9, Appendix A). These two species of insect also had the most visits to individual plants. The strongest interaction in the network was between *F. argentea* and *Anticlea elegans* Pursh (Melanthaceae) with 53 total visits. The second strongest interaction in the network was between *Bombus* sp. and *Centaurea stoebe* L. (Asteraceae) with 50 total visits (Table 7; Appendix A). Both these insects also visited *C. pitcheri* flowering heads (Table 8).

Pitcher's thistle had a total of 22 (37.3%) insect visitors and 109 (18.2%) visits out of the whole network. The two species with the second greatest number of visitors (11, 18.6%) were shrubby cinquefoil (*Dasiphora fruticosa* [L.] Rydb., Rosaceae) and the Lake Huron tansy (*Tanacetum bipinnatum* [L.] Sch. Bip. ssp. *huronense* (Nutt.) Breitung, Asteraceae). Spotted knapweed (*C. stoebe*) had the second greatest number of visits (68, 11.6%), roughly 60% of Pitcher's thistle visits (Table 7, Fig. 9). Pitcher's thistle also had a wider variety of insect visitors than next plants with the most visitors, with representatives from each of the six orders in the whole network (Table 8, Appendix A). Of the 22 visitors of Pitcher's thistle observed, there were six bee species and two ants (Hymenoptera), five flies (Diptera), three beetles (Coleoptera), three butterflies (Lepidoptera), two true bugs (Hemiptera) and *Trimerotropis huroniana* E. M. Walker (Lake Huron locust; Acrididae, Orthoptera) a federally endangered insect (Rankin and Crispin, 1994; Table 8).

Nearly all the species level network indices showed Pitcher's thistle to be the most topologically important plant in this network (ND, SS, BW, C and CW; Table 9). The SS value of 9.525 is 61% larger than the next largest SS value of 5.65 for *D. fruitcosa.* The index *d'*, a measure of species specialization,

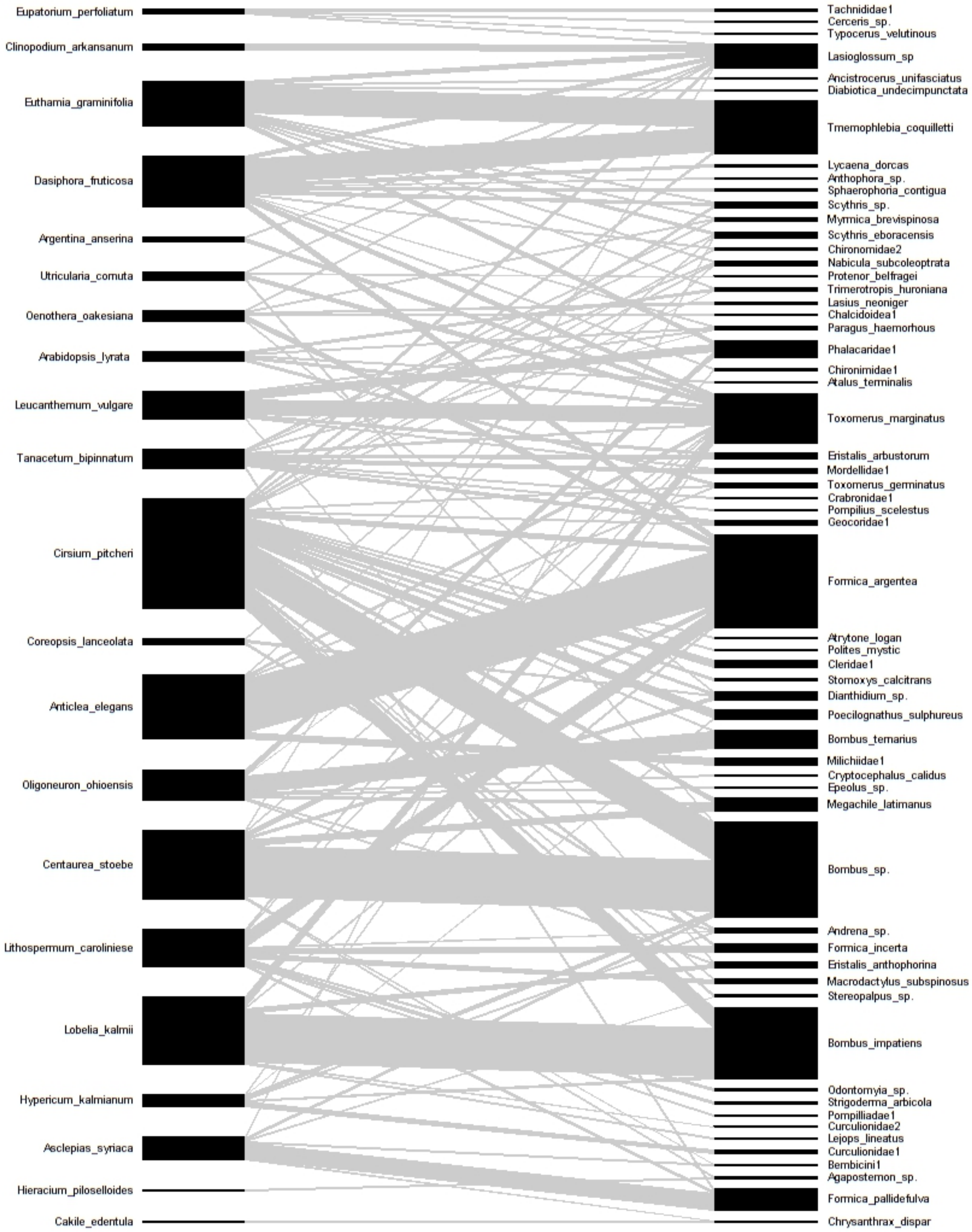

Fig. 9: The Sturgeon Bay dunes plant-insect visitor network as sampled during the *Cirsium pitcheri* flowering season in summer 2015. The right hand bars represent the insect visitors; the left represent the plants. Width of the respective bars depicts the total number of visits performed or number of visits received for insects and plants, respectively. The width of each gray bar shows the strength of the connection between each pair of plants and insects.

Table 7. Counts and percentages of insect species visitors (species richness) and total number of visits to each plant taxon in the Sturgeon Bay dune flowering plant-insect visitor network. Insect species percentages were derived from the 59 total insect taxa in the network. Insect visits percentages were computed from the 600 total observed.

Plant	Abbreviation	Insect Species		Insect Visits	
		Number	Percent	Number	Percent
Cirsium pitcheri	CIPI	22	37.3	109	18.2
Dasiphora fruticosa	DAFR	11	18.6	50	8.3
Tanacetum bipinnatum ssp. *huronense*	TABI	11	18.6	19	3.2
Euthamia graminifolia	EUGR	10	17.0	44	7.3
Lithospermum caroliniese	LICA	10	17.0	37	6.2
Centaurea stoebe	CEST	8	13.6	68	11.3
Oenothera oakesiana	OEOA	6	10.2	11	1.8
Oligoneuron ohioensis	OLOH	8	13.6	30	5.0
Lobelia kalmii	LOKA	6	10.2	66	11.0
Leucanthemum vulgare	LEVU	6	10.2	27	4.5
Hypericum kalmianum	HYKA	6	10.2	12	2.0
Anticlea elegans	ANEL	5	8.5	64	10.7
Asclepias syriaca	ASSY	6	10.2	23	3.8
Arabidopsis lyrata	ARLY	5	8.5	9	1.5
Utricularia cornuta	UTCO	4	6. 8	8	1.3
Eupatorium perfoliatum	EUPE	4	6. 8	5	0.8
Coreopsis lanceolata	COLA	3	5.1	5	0.8
Argentina anserina	ARAN	2	3.4	5	0.8
Clinopodium arkansanum	CLAR	1	1.7	6	1.0
Cakile edentula	CAED	1	1.7	1	0.2
Hieracium piloselloides	HIPI	1	1.7	1	0.2

Table 8. Insect visitors and number of visits to *Cirsium pitcheri* in the Sturgeon Bay dune plant-insect flower visitor network surveyed during the 2015 *C. pitcheri* flowering season.

Species/Morphospecies	Family	Order	Visits
Bombus sp.	Apidae	Hymenoptera	37
Bombus impatiens	Apidae	Hymenoptera	20
Poecilognathus sulphureus	Bombyliidae	Diptera	8
Cleridae1	Cleridae	Coleoptera	7
Formica argentea	Formicidae	Hymenoptera	5
Lasioglossum sp.	Halictidae	Hymenoptera	4
Megachile latimanus	Megachilidae	Hymenoptera	4
Formica incerta	Formicidae	Hymenoptera	3
Stomoxys calcitrans	Muscidae	Diptera	3
Dianthidium sp.	Megachilidae	Hymenoptera	2
Tmemophlebia coquilletti	Bombyliidae	Diptera	2
Scythris sp.	Scythrididae	Lepidoptera	2
Bombus ternarius	Apidae	Hymenoptera	2
Stereopalpus sp.	Anthicidae	Coleoptera	2
Nabicula subcoleoptrata	Nabidae	Hemiptera	1
Toxomerus germinatus	Syrphidae	Diptera	1
Trimerotropis huroniana	Acrididae	Orthoptera	1
Macrodactylus subspinosus	Scarabidae	Coleoptera	1
Geocoridae1	Geocoridae	Hemiptera	1
Chironomidae2	Chironomidae	Diptera	1
Atrytone logan	Hesperiidae	Lepidoptera	1
Polites mystic	Hesperiidae	Lepidoptera	1

Table 9. Species level network indices for the Sturgeon Bay dune flowering plant-insect visitor network. The indices are normalized degree (ND), species strength (SS), betweenness (B), weighted betweenness (BW), closeness (C), weighted closeness (CW) and *d’*.

Plant	ND	SS	B	BW	C	CW	*d'*
Cirsium pitcheri	0.373	9.525	0.078	0.310	0.055	0.029	0.362
Dasiphora fruticosa	0.186	5.366	0.038	0.056	0.053	0.028	0.505
Tanacetum bipinnatum	0.186	5.186	0.044	0.004	0.054	0.019	0.661
Euthamia graminifolia	0.169	4.559	0.003	0.003	0.052	0.028	0.626
Lithospermum caroliniese	0.169	5.620	0.076	0.076	0.052	0.027	0.537
Centaurea stoebe	0.136	2.482	0.299	0.209	0.058	0.028	0.554
Oenothera biennis	0.102	2.557	0.028	0.000	0.049	0.018	0.471
Oligoneuron ohioensis	0.136	3.860	0.014	0.000	0.050	0.024	0.759
Lobelia kalmii	0.102	3.578	0.005	0.119	0.049	0.029	0.686
Leucanthemum vulgare	0.102	1.785	0.048	0.048	0.047	0.029	0.603
Hypericum kalmianum	0.102	1.392	0.034	0.000	0.051	0.016	0.404
Anticlea elegans	0.085	2.094	0.008	0.043	0.050	0.033	0.704
Asclepias syriaca	0.102	2.293	0.212	0.132	0.043	0.023	0.722
Arabidopsis lyrata	0.085	2.311	0.004	0.000	0.049	0.016	0.627
Utricularia cornuta	0.068	1.146	0.075	0.000	0.055	0.018	0.317
Eupatorium perfoliatum	0.068	3.043	0.000	0.000	0.041	0.011	0.765
Coreopsis lanceolata	0.051	0.817	0.010	0.000	0.043	0.009	0.698
Argentina anserina	0.034	0.125	0.038	0.000	0.053	0.016	0.378
Clinopodium arkansanum	0.017	0.261	0.000	0.000	0.041	0.019	0.669
Cakile edentula	0.017	0.500	0.000	0.000	0.026	0.005	0.848
Hieracium piloselloides	0.017	0.500	0.000	0.000	0.031	0.005	0.848

indicates that Pitcher's thistle is the most generalized (0.362) plant taxon. The two species with only one visit (*Cakile edentula* (Bigelow) Hook., Brassicaceae; *Hieracium piloselloides* Vill., Asteraceae) were the most specialized (0.864). Betweenness (B) showed *Asclepias syriaca* L. (Apocynaceae) as the most topologically important species; however, when the index was weighted by number of visits (BW), Pitcher's thistle far exceeded *A. syriaca* (Table 9).

C. pitcheri had many more visits relative to its abundance on the landscape than did other plants present (Fig. 9). There were 123 open flower heads on a total of 44 Pitcher's thistle plants observed during the entire sampling period, yet *C. pitcheri* still received the most visits by insects. In contrast, Carolina poccoon, *Lithospermum caroliniense* (Walter ex J.F. Gmel.) MacMill. (Boraginaceae), had 1,874 flowers on 54 plants, over 15 times the number of flowers of Pitcher's thistle, yet received only 37 total visits from insects (Fig. 10A, Table 7). The plant that received the most total observation time was *Lobelia kalmii* L. (Campanulaceae) with 4,390 min (439 plants). Despite this abundance, *L. kalmia* received 60.5% (66) as many visits as Pitcher's thistle (Fig. 10B, Table 7).

3.4 DISCUSSION

Cirsium pitcheri is an important floral resource for insect visitors. Pitcher's thistle had many more visits and types of insect visitors than did any other plant species in the Sturgeon Bay dune ecosystem. The species-level network metrics also point to Pitcher's thistle as a possible key species supporting the dune network structure. Betweenness and Closeness are measures of network centrality, or how much each member contributes to network structure. The high BW and CW scores that I observed demonstrate that Pitcher' thistle may mediate insect visits to other plant species. *C. pitcheri* was the most generalized plant in the dune network (*d'*, Table 9), receiving insect visits from the six insect orders in the network (Table 8). Generalists are usually very important species that maintain network structure (Sahli and Conner, 2006) and also are more likely to be considered keystone species in these same networks (Martín González et al., 2010; Pocock et al., 2011).

The modern definition of a keystone species states that the effect of a keystone species is disproportionately large relative to abundance of that species (Power et al., 1996). In the plant-insect visitor network literature, network analysis species-level indices typically are used to identify topologically

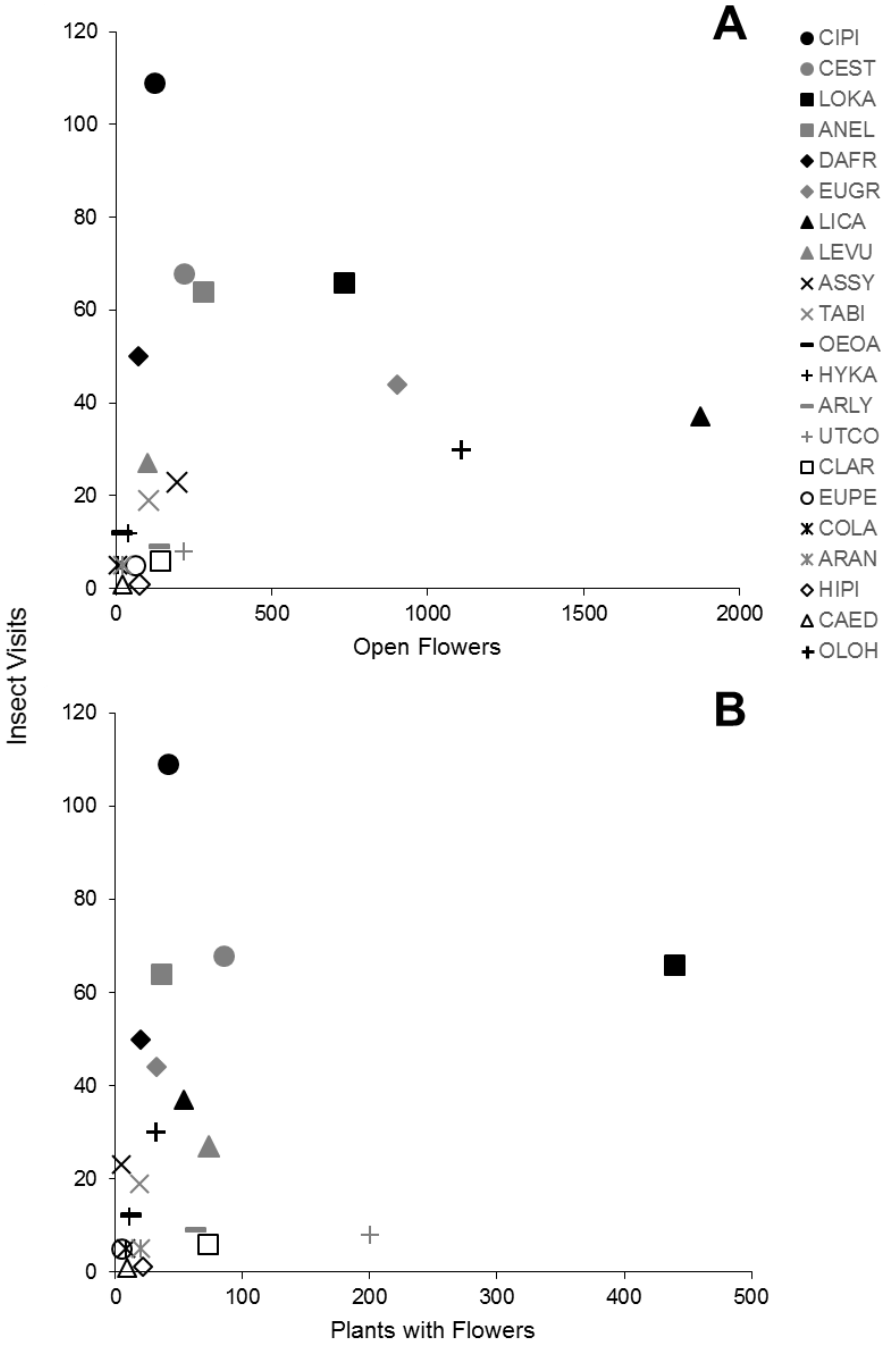

Fig. 10. Number of insect visits each species received relative to abundances of the A) the total number of open flowers and B) the total number of plants that were observed for insect visits in the dune network. The legend names correspond to the first two letters of the genus and specific epithet of the plant taxa.

important species, but these indices do not take into account relative abundance (Robson, 2014; Koski et al., 2015). In Fig. 9, I show that *Cirsium pitcheri* has many insect visits relative to abundance of flowers and reproductive plants in the network. There is a distinct gap in the number of insect visits between *C. pitcheri* and other plants species with similar abundances (Fig. 10). An unknown Cleridae beetle (Coleoptera; 7 visits), *Stomoxys calcitrans* (Muscidae, Diptera; 3 visits), *Atryone logan* (Hesperidae, Lepidoptera; 1 visit) and *Polites mystic* (Hesperidae, Lepidoptera; 1 visit) visited Pitcher's thistle exclusively (Appendix A). Loss of Pitcher's thistle could result in extinction of these insect species in the Sturgeon Bay dune ecosystem, if these insects are unable to switch resources.

Though Pitcher's thistle is disproportionately visited relative to abundance of its floral resources, these flowering units (the head or capitulum inflorescence) offer greater amounts of pollen and nectar to insect visitors. The floral displays of *Lithospermum caroliniense or Lobelia kalmia* are much smaller than the capitula of *C. pitcheri*. Other plant-pollinator networks also use inflorescences of plant families Asteraceae and Apiaceae as the flowering unit for quantification; insects do not visit single florets, but rather whole inflorescences or heads (Memmott et al., 1999). Few plant-pollinator network studies compare resource availability (pollen, nectar) among species. An obvious next step is to document resource availability, including energy and nutrient flow through ecosystems, as possible mechanisms to explain network structure.

Not only are plants important for insect flower visitors, but for other interactions as well. Mistletoe in eucalyptus forests in Australia was shown to be a keystone species after a removal experiment. The mistletoe removal plots showed significant reduction of frugivores, mistletoe specialist herbivores and woodland-dependent bird species richness compared to control plots (Watson and Herring, 2012). Few papers claiming keystone status of species use removal to examine the effect of an individual on an ecosystem, likely due to the impracticality of removal. It is not only impractical to remove reproductive Pitcher's thistle plants from the dune ecosystem to demonstrate keystone importance, but also illegal as this plant is protected under the Endangered Species Act (16 U.S.C. § 1531-1544, 1973).

Another example of a keystone interaction relates to the phenology of the plant's available resources. Fig species (*Ficus* sp., Moraceae) in tropical rainforests of southeastern Asia support frugivore animals at times of year when other fruits are scarce, providing critical nutritional resources for many vertebrate species (Lambert and Marshall, 1991). Pitcher's thistle flowers late-June to late-July after abundant spring floral resources such as bearberry (*Arctostaphylous uva-ursi* L., Ericaceae) and sand cherry (*Prunus pumila* L. Rosaceae) and before fall flowering resources such as Houghton's goldenrod (*Oligoneuron houghtonii* [Torr. & A.Gray ex A.Gray] G.L.Nesom, Asteraceae) and Ohio goldenrod (*Oligoneuron ohioensis* [Frank ex Riddell] G.N. Jones, Asteracea) (Voss, 1972; Fig. 8). My network analysis does not attempt to analyze the importance of the dune floral resources through the entire growing season but instead quantifies the relative importance of plants during the Pitcher's thistle flowering period.

Pitcher's thistle is not the only threatened or narrowly endemic plant species that is ecologically important. *Ceroxylon echinulatum* Galeano (Arecaceae) is a locally abundant threatened palm endemic to the cloud forests of Ecuador and Peru. In its native habitat, *C. echinulatum* is an important resource for seed and fruit eating animals, but it is threatened by habitat fragmentation and deforestation (Anthelme et al., 2011). *Anchusa littorea* Moris (Boraginaceae) is a coastal endemic of the island Sardinia, Italy, was considered extinct before rediscovery in 2008. *A. littorea* now serves as key indicator for dune habitat degradation due to human trampling in the found populations (Fenu et al., 2013).

Future studies should explore the relative importance of Pitcher's thistle in more degraded and less diverse ecosystems. Basket et al. (2011) showed that insect diversity and visits to Pitcher's thistle increased in areas after invasive species such as baby's breath (*Gysophilla paniculata* L., Caryophyllaceae) and spotted knapweed (*C. stoebe*) were removed. In the Sturgeon Bay dune system, similar Hymenoptera visited Pitcher's thistle and spotted knapweed extensively (Appendix A). With the removal of knapweed, we would expect to see increased and stronger visitation relationships of Hymenopterans on Pitcher's thistle. I observed many of the same insect visitors to Pitcher's thistle as have others, including the same members of the families Apidae (*Bombus impatiens*), Bombyliidae, Formicidae, Halictidae (*Lasioglossum* sp.), Hesperidae, Megachilidae (*Megachile latimanus*), Nymphalidae, Scarabidae (*Strigoderma arbicola*) (Keddy and Keddy, 1984; Loveless, 1984; Baskett et al., 2011). The consistency of similar insect visitors to Pitcher's thistle across time and space demonstrates the potential of this plant as an important floral resource in other dune and cobble shore habitat throughout the Pitcher's thistle range.

Pitcher’s thistle populations suffer many threats including coastal habitat degradation (Rankin and Crispin, 1994), herbivory (Phillips and Maun, 1996; Stanforth et al., 1997; Bevill et al., 1999; Rowland and Maun, 2001;), low germination in the field (Hamzé and Jolls, 2000), low genetic diversity (Gauthier et al., 2010), invasive species encroachment (Baskett et al. 2011; Emery et al., 2013), climate change-induced habitat narrowing (Vitt et al. 2010), and most recently biological control non-target impacts (Chapter 2; Havens et al., 2012; Havens et al., 2014; Havens et al., 2015). Despite these threats, this study has shown that in relatively pristine Great Lakes dune habitats, Pitcher’s thistle is an important floral resource for flower visiting insects. The loss of this potential keystone species may destabilize the whole network, leading to a cascade of secondary extinctions (Memmot et al., 2004). Efforts should be made to quantify Pitcher’s thistle floral resources in other Great Lakes dune habitats and to search for solutions to threats.

3.4.1 Conclusions

Pitcher’s thistle is an important floral resource for the insect species visitors in the dune network. More insect species visit this plant than any other in the network. Network analysis species-level indices also show Pitcher’s thistle is topologically important for the network structure. Abundance of *C. pitcheri* is low compared to other plants in the network, yet it is visited by disproportionately greater abundances of insect visitors compared to the other plants. Pitcher’s thistle may function as a keystone species in the dune network for floral insect visitors in mid-summer, increasing the importance of conservation efforts of this plant species.

CHAPTER 4: OVERVIEW AND GENERAL CONCLUSIONS ABOUT THE ANTAGONISTIC AND MUTUALIST INSECT INTERACTIONS OF PITCHER'S THISTLE

Pitcher's thistle is a federally threatened, Great Lakes dune and cobble shore endemic species. There are many threats to this species including habitat degradation and range narrowing (Rankin and Crispin, 1994; Vitt et al., 2010; Emery et al., 2013), natural herbivory (Phillips and Maun, 1996), low genetic diversity (Gauthier et al., 2010) and limited life history (Loveless 1984; Hamzé and Jolls, 2000). One of the more recent threats is from non-target impacts by a biological control agent (Havens et al., 2012).

Biological control is one of the tools used by land managers for integrated pest management of invasive plant species. One of the major drawbacks of this technique is the risk of non-target impacts of the biocontrol agent on native species. An example of non-target impacts is the interaction between the seed head weevil *Larinus planus* and the Great Lakes dune and cobble shore endemic threatened thistle, *Cirsium pitcheri*. I repeatedly surveyed 44 Pitcher's thistle plants for impacts from *L. planus* from three populations in northern lower Michigan from mid-June to mid-August 2015. I found that reproductive life cycle of *L. planus* overlaps with the early reproductive phase of the Pitcher's thistle. The insect oviposits on thistle heads from mid-June to early July, before the heads flowered. Virtually all oviposition ended by mid-July (Fig. 3). The heads that received oviposition had a narrow size range, on average 12-14 mm in diameter (Table 1), and the majority of heads oviposted on were less than 15 mm in diameter (Fig. 4). A generalized linear mixed binary logistic model reported date of oviposition and size of heads as significant predictors of oviposition on heads (Table 4). Approximately 32% of the 1,695 heads surveyed at all three sites had oviposition. A subset of heads with oviposition were dissected to determine weevil survival. I found that 44% of the time, a weevil egg survived to damage seeds (Table 2).Thus, approximately 14% of heads surveyed showed seed damage by weevils. When a weevil developed, the number of filled seeds (most likely to be viable and capable of producing a seedling) were reduced by 62% (Fig. 5). Other studies that examined the effect of non-target impacts of biological control weevils have found similar levels of impact of *L. planus* on another native thistle in Wyoming (DePrenger-Levin et al., 2010) and also that phenology of the host plant was an important contributor of weevil impact on individual plants and plant populations (Russel and Louda, 2005).

Control of *L. planus* is essential for persistence of *C. pitcheri* populations. I tested the effectiveness of a certified organic insect deterrent Surround® WP in reducing *L. planus* impacts on *C. pitcheri*. This product is primarily made of kaolin clay which is mixed in water and then applied to the plant to create a protective film after drying. I found that kaolin clay applied to flowering Pitcher's thistle heads did not deter insect pollinator visits, reduce species richness of visiting insects or change the length of the visit. I applied either kaolin clay or water (control) to pairs of heads on 18 Pitcher's thistle plants. I found that the clay-treated heads had significantly fewer *L. planus* oviposition holes and were less likely to have oviposition at all compared to water-treated heads (Fig. 6, Table 6). On average there were significantly more filled seeds per head treated with kaolin clay than water-treated heads (Fig. 7). Most of the other studies that tested kaolin clay effectiveness in reducing insect impacts on plants were from agricultural applications. Kaolin clay reduced but did not eliminate oviposition of a root weevil on citrus plants compared to a water control (Lapointe et al., 2006). Kaolin clay also reduced impacts from the boll weevil on cotton plants, but endosulphan insecticide was yet more effective still (Silva and Ramalho, 2013). Kaolin clay may indirectly increase seed set by deterring weevils, but also may directly increase seed set through effects of temperature due to the high reflective albedo of the particle film (Tessenderlo Kerley, 2008c). Leaf tissue of olive trees had lower temperatures with kaolin clay applied compared to a water control, effectively preventing drought stress (Denaxa et al., 2012). This temperature and drought-reducing quality of this product may have fostered the greater number of filled seeds in kaolin clay-treated heads.

Depending on the size of Pitcher's thistle populations and spread of the weevil infestation, the use of kaolin-clay may be very cost effective. One 11.3 kg (25 lbs) bag of Surround® WP costs roughly 50 USD and can be used to make 378L (100 gal) of deterrent spray. Not all reproductive phase Pitcher's thistle plants are attacked by the weevil and all plants will require repeated treatments. The most expensive part of the treatment would be human labor for application of the deterrent. Application of kaolin clay, however, is only needed during the relatively narrow time frame of impacts during weevil oviposition.

I have several recommendations for land managers monitoring populations of Pitcher's thistle. I recommend applying the aqueous solution of kaolin clay to Pitcher's thistle plants early in the overlap of the reproductive phases of Pitcher's thistle and the seed head weevil, mid-June to early July. Application should occur every 3-7 days depending on precipitation and wind patterns. Kaolin clay should be applied to heads that are not yet flowering. Application of kaolin clay is typically through a drench, dip or backpack sprayer. Use of a backpack sprayer should confine clay applications to *C. pitcheri* heads only. Experiments are needed to confirm that extensive applications to other parts of the plant body do not affect photosynthetic rates or growth. Reduction of the impact of this weevil is not only critical for conservation of this species, but may be important for the entire dune plant-insect flower visiting network.

Cirsium pitcheri may be an important floral resource for many flower visiting insects in the dune network. Pitcher's thistle flowers for short period of time in the middle of the summer months, late-June to early August. During this time, many species are at the end of their late spring flowering period or just beginning to flower in the early fall (Fig. 8; Voss, 1972; Goodwillie and Jolls, 2013). Many different species of insect from several taxonomic orders visit Pitcher's thistle flower heads indicating a generalist pollination syndrome (Keddy and Keddy, 1984; Loveless, 1984; Basket et al., 2011). Generalists plants are often important floral resources and help maintain plant-pollinator network structure (Sahli and Conner, 2006). I hypothesized that Pitcher's thistle would be an important floral resource for the dune flower visiting insects relative to the other floral resources and abundance of those resources, during the *C. pitcheri* flowering season in 2015. To test this hypothesis I performed insect visitor observations on all the insect pollinated plants in randomly selected plots in the dunes of Sturgeon Bay, Wilderness State Park, MI. I recorded insect species visits, number of visits and the number of open flowers per plant observed. The dune plant-insect flower visitor network consisted of 21 plant species and 59 insect species (in 6 taxonomic orders) with 600 total visits recorded over 233.5 hours of observation. I used R Bipartite package to estimate the importance of individual plants in the whole network.

I demonstrated that Pitcher's thistle is indeed an important floral resource for the insect flower visitors. *C. pitcheri* received the most visits and had the most visitor species compared to any other plant in the network, by a large margin (Table 7). Pitcher's thistle also had the consistently highest species-level network metrics such as species strength (sum of visitor dependencies on the plant), and Connectedness and Betweenness (measures of centrality in maintaining network structure). The index *d'* (a measure of a species specificity) was much lower for Pitcher's thistle compared to other plants, confirming that Pitcher's thistle, like many Asteraceae, has a generalist pollination syndrome (Table 7). Pitcher's thistle also had much lower abundance relative to the number of visits received. The species with the highest abundance of open flowers or plants had ≤ 40% fewer visits. I concluded that Pitcher's thistle has a disproportionate effect on the flower visiting insect dune fauna, relative to the abundance of the plant's floral resources. I concluded that Pitcher's thistle was the plant in the dune network that was most likely to be considered a keystone species in the middle summer months. Other plants have been classified as a keystone species based on the diversity and frequency of insect visits to floral resources (Robson, 2014; Koski et al., 2015). These studies did not consider plant abundance as a factor in determining keystone status as did my study.

Conservation of Pitcher's thistle should be an important conservation priority in the freshwater dunes and cobble shores of the Great Lakes. Loss of this species due to threats such as the non-target biological control weevil could result in a cascade of secondary extinctions, and a much less robust dune plant-insect network.

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APPENDIX A

Part 1 of 4: Insect species (upper row) and the frequency of visits to plants (left column) in the plant-insect visitor network.

Visited Plant Species	*Toxomerus marginatus*	*Lasioglossum* sp.	*Formica argentea*	*Bombus* sp.	*Dianthidium* sp.	*Scythris eboracensis*	*Nabicula subcoleoptrata*	*Andrena* sp.	*Paragus haemorhous*	*Bombus impatiens*	*Tmemophlebia coquilletti*	*Formica pallidefulva*	*Megachile latimanus*	Phalacaridae1	*Eristalis anthophorina*
Cirsium pitcheri	0	4	5	37	2	0	1	0	0	20	2	0	4	0	0
Dasiphora fruticosa	4	4	6	0	0	0	0	0	1	0	24	0	0	0	0
Tanacetum bipinnatum ssp. *huronense*	2	0	0	0	0	1	1	0	1	0	0	2	0	0	0
Euthamia graminifolia	0	4	0	0	0	3	2	0	0	0	26	0	0	4	0
Lithospermum caroliniense	1	0	15	0	0	0	0	1	0	0	0	3	0	0	0
Centaurea stoebe	1	1	5	50	3	0	0	0	0	0	0	0	6	0	0
Oenothera oakesiana	0	1	5	0	1	0	0	0	0	0	0	0	0	0	0
Oligoneuron ohioensis	1	0	0	0	0	0	0	1	0	0	0	0	3	6	1
Lobelia kalmii	10	0	2	0	0	0	0	0	0	48	0	0	0	0	4
Leucanthemum vulgare	15	0	0	0	0	0	0	0	1	0	0	0	0	7	1
Hypericum kalmianum	1	0	0	3	0	0	0	1	0	2	0	0	0	0	0
Anticlea elegans	3	0	53	0	0	0	1	0	0	0	0	0	0	0	0
Asclepias syriaca	0	0	0	3	0	1	0	0	0	0	0	16	0	0	0
Arabidopsis lyrata	3	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Utricularia cornuta	4	1	0	2	0	0	0	0	0	0	0	0	0	0	0
Eupatorium perfoliatum	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Coreopsis lanceolata	0	0	0	0	2	1	0	2	0	0	0	0	0	0	0
Argentia anserina	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Clinopodium arkansanum	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0
Cakile edentula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hieracium piloselloides	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Plants Visited	12	9	7	5	4	4	4	4	4	3	3	3	3	3	3
Total Visits	49	23	91	95	8	6	5	5	4	70	52	21	17	13	6

Part 2 of 4: Insect species (upper axis) and the frequency of visits to plants (left axis) in the plant-insect visitor network.

Plant sp./Insect sp.	*Scythris* sp.	*Toxomerus germinatus*	*Trimerotropis huroniana*	*Myrmica brevispinosa*	*Bombus ternarius*	*Poecilognathus sulphureus*	*Formica incerta*	*Macrodactylus subspinosus*	*Eristalis arbustorum*	Mordellidae1	Geocoridae1	*Stereopalpus* sp.	Chironimidae1	Chironomidae2	*Agapostemon* sp.
Cirsium pitcheri	2	1	1	0	2	8	3	1	0	0	1	2	0	1	0
Dasiphora fruticosa	3	0	0	0	0	0	0	0	0	0	0	0	1	1	0
Tanacetum bipinnatum ssp. *huronense*	0	0	0	1	0	0	0	0	3	3	3	0	0	0	0
Euthamia graminifolia	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0
Lithospermum caroliniense	0	0	0	0	0	0	5	4	0	0	0	0	2	0	0
Centaurea stoebe	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Oenothera oakesiana	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Oligoneuron ohioensis	0	0	0	0	16	0	0	0	0	0	0	0	0	0	0
Lobelia kalmii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leucanthemum vulgare	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0
Hypericum kalmianum	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Anticlea elegans	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Asclepias syriaca	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Arabidopsis lyrata	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0
Utricularia cornuta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eupatorium perfoliatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coreopsis lanceolata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Argentia anserina	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Clinopodium arkansanum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cakile edentula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hieracium piloselloides	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Total Plants Visited	3	3	3	3	2	2	2	2	2	2	2	2	2	2	2
Total Visits	6	4	4	3	18	9	8	5	5	4	4	3	3	2	2

Part 3 of 4: Insect species (upper axis) and the frequency of visits to plants (left axis) in the plant-insect visitor network.

Plant sp./Insect sp.	Bembicini1	Cleridae1	Milichiidae1	Curculionidae1	*Strigoderma arbicola*	*Stomoxys calcitrans*	*Lycaena dorcas*	*Sphaerophoria contigua*	Tachnididae1	*Odontomyia* sp.	*Lasius neoniger*	*Atalus terminalis*	*Chrysanthrax dispar*	*Atrytone logan*	*Polites mystic*
Cirsium pitcheri	0	7	0	0	0	3	0	0	0	0	0	0	0	1	1
Dasiphora fruticosa	0	0	0	0	0	0	3	2	0	0	0	0	0	0	0
Tanacetum bipinnatum ssp. *huronense*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Euthamia graminifolia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lithospermum caroliniense	0	0	0	0	3	0	0	0	0	2	0	0	0	0	0
Centaurea stoebe	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oenothera oakesiana	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
Oligoneuron ohioensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lobelia kalmii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leucanthemum vulgare	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hypericum kalmianum	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0
Anticlea elegans	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0
Asclepias syriaca	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Arabidopsis lyrata	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Utricularia cornuta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eupatorium perfoliatum	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
Coreopsis lanceolata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Argentia anserina	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Clinopodium arkansanum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cakile edentula	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Hieracium piloselloides	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Plants Visited	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1
Total Visits	2	2	7	6	4	3	3	3	2	2	2	2	1	1	1

Part 4 of 4: Insect species (upper axis) and the frequency of visits to plants (left axis) in the plant-insect visitor network.

Plant sp./Insect sp.	*Anthophora* sp.	*Cerceris* sp.	*Typocerus velutinous*	*Ancistrocerus unifasciatus*	*Diabiotica undecimpunctata*	Pompilliadae1	Curculionidae2	*Lejops lineatus*	Chalcidoidea1	*Cryptocephalus calidus*	*Epeolus* sp.	Crabronidae1	*Pompilius scelestus*	*Protenor belfragei*
Cirsium pitcheri	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dasiphora fruticosa	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Tanacetum bipinnatum ssp. *huronense*	0	0	0	0	0	0	0	0	0	0	0	1	1	0
Euthamia graminifolia	0	0	0	1	1	0	0	0	0	0	0	0	0	0
Lithospermum caroliniense	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Centaurea stoebe	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oenothera -oakesiana	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Oligoneuron ohioensis	0	0	0	0	0	0	0	0	0	1	1	0	0	0
Lobelia kalmii	0	0	0	0	0	0	1	1	0	0	0	0	0	0
Leucanthemum vulgare	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hypericum kalmianum	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anticlea elegans	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Asclepias syriaca	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arabidopsis lyrata	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Utricularia cornuta	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Eupatorium perfoliatum	0	1	1	0	0	0	0	0	0	0	0	0	0	0
Coreopsis lanceolata	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Argentia anserina	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Clinopodium arkansanum	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cakile edentula	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hieracium piloselloides	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Plants Visited	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Total Visits	1	1	1	1	1	1	1	1	1	1	1	1	1	1