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The Weevil Next Door: Exploring the impact of associational effects on *C. pitcheri* to better biological control practices

Tina Czaplinska

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The Weevil Next Door

Exploring the impact of associational effects on *C. pitcheri* to better biological control practices

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Lake Michigan dwelling dune thistle, *Cirsium pitcheri*, is a federally threatened plant, whose reproduction is affected by a non-native weevil, *Larinus planus*. Originally introduced as a biological control agent to combat the spread of Canada thistle, *Cirsium arvense*, this weevil is instead using *C. pitcheri* as a host for its larvae. Associational susceptibility is an important factor to consider in hopes of preventing the damage to this endangered plant. The goal of this study was to observe *L. planus* behavior to determine why there is a correlation between increased density of beach grass and high levels of *C. pitcheri* damage. We hypothesize that *C. pitcheri*'s neighboring grass community is used as a dispersal aid for the non-native weevil, making neighboring thistle hosts more susceptible to weevil damage in grassy environments. To test this, we conducted ethogram studies at Whitefish Dunes State Park (WDSP) in Door County, WI. At WDSP, we found that *L. planus* physically used beach grass to get to *C. pitcheri* and largely failed to disperse using the sand. These results should help provide ecologically sustainable management strategies, while also promote more in-depth host specific analyses prior to the release of biological control agents.

Understanding insect behavior can often be the first step to plant conservation. In this study, we observe how weevil dispersal behavior is affected by indirect associational mechanisms and abiotic factors. Through this, we hope to gain a better understanding of insect pest management, in order to conserve plants that are subject to their herbivory.

Biological Control Overview

Implementing non-native species for biological control (biocontrol) purposes has earned a divided reputation. At first, biological control was not met with such contention. Redeeming qualities of biocontrol include cost effectiveness, sustainability and that it is environmentally sound. Successfully established populations of biocontrol agents require only a singular cost of introduction. The first well-publicized and successful means of biological control was the 1888 introduction of the vedalia beetle, *Rodolia carinalis*, into California to manage the cottony cushion scale, *Icerya purchasi* (Doutt, 1988). Subsequently, numerous other successful biocontrol introductions have been performed, most notably was that involving the control of the purple loosestrife (*Lythrum salicaria*). In the ten years since the release of two leaf beetles, *Galerucella californiensis* and *Galerucella pusilla*, reduced flowering frequency has been one of the consistent results of beetle feeding (Grevstad, 2006). In most published studies, purple loosestrife plants were found to be shorter on average, sustaining various levels of beetle damage (Grevstad, 2006; Lindgren, 2003; Denoth *et al.* 2005; Blossey *et al.* 2000).

Victories like these vary in their implementation but are rooted in a similar, general process.

Biocontrol agent invasion is successful after a series of stages are completed: uptake, transport, release, introduction and establishment (Colautti *et al.* 2004). This success is typically attributed to the population's release into an environment free from the effects of their predators. The degree of the success of this non-native species invasion is explained by the enemy-release hypothesis (ERH), which suggests that the abundance or impact of some non-native species is related to the scarcity of natural enemies in the introduced range as compared to the native range (Keane *et al.* 2002). As a result, biocontrol agents highlight the potential importance of natural predators in preserving ecological balance. Consequently, introduced agent populations grow larger in introduced ranges than in their native range (Crawley 1987). However, even biocontrol agents that are deemed successful may be negatively affected by other processes that are unrelated to enemy release, like environmental variables, human activity, selection for invasive genotypes etc (Keane *et al.* 2002).

Different biocontrol programs have yielded successful results in reducing pest populations. In their 2005 meta-analysis of biocontrol programs, Stiling and colleagues gathered 145 independent studies. Their goal was to examine the efficacy of different biocontrol agents in reducing the abundance and performance of several weed and pest species. In sum, their data show that non-target effects were much smaller than effects on target species, which supports the continued use of biocontrol. The feeding mode and specialization of the biocontrol agent may also play a role in its success or failure.

For example, the team observed significant effects of biocontrol programs involving folivores, pathogens and sap feeders on target weeds. Other studies also supported the continued use of biocontrol programs, finding that only 10% or less of classical biocontrol programs have led to population changes in non-target organisms (Lynch *et al.* 2001). Similarly, there has been a push for multi-species releases of biocontrol agents in hopes of reducing pest populations (Stiling *et al.* 2005). Although inter-specific competition and intra-guild predation are among the interactions that might disturb effective biocontrol of pests (van den Bosch, 1971), multiple agents were found to decrease pest abundance by 27.2% compared to single species releases (Stiling *et al.* 2005). This is certainly reinforced by the famously successful case of the purple loosestrife, which has four biocontrol agents released in Wisconsin and Minnesota. This approach typically functions under the implicit bias that the information available on the biocontrol agents prior to release is sufficient to predict and therefore prevent unwanted ecological outcomes.

While biological control has been considered an important component of integrated pest management (Waage *et al.* 1988), some researchers have highlighted the dangers of this route, finding that the benefits do not outweigh the risks (Simberloff *et al.* 1996). Potential harmful effects, varying from endangering to extinguishing non-target species, stress the inadequacies surrounding the implementation of biological control. For example, after the victory over the cottony cushion scale led to haphazard releases of predatory insects that failed to yield positive results. Instead of eliminating a pest, the introduced enemy becomes a pest itself. Success rates vary, with 41% of cases

resulting in some control and 20% of cases with in complete control (Louda *et al.* 2003). This firstly suggests that native relatives of targeted species are most vulnerable to predation by biocontrol agents. For example, a type of predatory snail, *Euglandina rosea*, has been introduced from Florida and Central America to many islands worldwide with the intention of controlling another snail, *Achatina fulica*, but has instead extinguished several endemic forest snails (Simberloff *et al.* 1996). Next, the data also suggests that the relationship between feeding preference and actual levels of herbivory is complicated by environmental context, which can influence the agent's choice. For example, the small Indian mongoose (*Herpestes auropunctatus*), introduced to a medley of island countries to control rats in agricultural fields, instead contributed to the decline of birds native to those areas (Simberloff *et al.* 1996). In general, any predator or herbivores maintained at high densities on common alternative hosts can potentially drive a rare non-target species to extinction (Simberloff *et al.* 1996).

Recent studies suggest that current biocontrol assumptions need to be reevaluated (Louda *et al.* 2003). Through this, the question is not if biocontrol programs can be effective, but how can they be made most effective. Namely, focusing on what pairing of biocontrol agent and pest is ideal for a given situation. When the pairings are incorrect, biocontrol fails. These seemingly predictable outcomes of success and failure are typically the product of limited quantitative information on most biological control programs (Greathead 1986). Currently, evaluations of biological control are typically based on non-quantitative economic criteria (Stiling *et al.* 2005). Inadequate analysis makes it difficult to establish a consistent measure of success for biological control

agents. Ideally, analysis would detail how life history, phenology and population parameters are correlated with known outcomes within a given environment, in order to minimize non-target effects.

Interestingly, although the merits of generalists over specialist in biocontrol efficacy were questioned in the past, more recent data have begun strengthening the case for generalists. Specialist refers to species that are able to thrive in specific environments and/or on a specific diet, whereas generalists can function in a wider breadth of environments and or diets. In a review using 181 manipulative studies, approximately 75% of the cases using generalist predators significantly decreased pest abundance (Stiling *et al.* 2005). When only studying the compatibility of the biocontrol agent and target host, unintended effects may inadvertently be ignored. However, associational susceptibility is not necessarily related to higher abundances of generalist herbivores. Instead, it can rely on one or more specialist herbivores (Plath *et al.* 2012). Despite this, researchers have concluded that it might not be possible to predict the impact of specific plant associations simply based on the diet breadth of the herbivore in question when only considering the relationship being a neighboring plant and a target plant (Barbosa, *et al.* 2009).

All of this, in combination, emphasizes the individuality of each ecological circumstance, revealing the need for individualized biocontrol treatment options.

Associational Susceptibility Impact

Plant-plant associations affect the probability that a plant will be vulnerable to herbivory. Plant associations can increase, known as associational susceptibility, or decrease, known as associational resistance, the likelihood of vulnerability to herbivores. In associational susceptibility, the outcome of this interaction is negative for a target plant, enhancing chances of detection and damage. Target plant damage is facilitated by a variety of factors, like palatability and taxonomy. Here, herbivory resulting from associational susceptibility typically involves herbivorous insects, not herbivorous mammals (Barbosa *et al.* 2009). Palatability of neighboring plants can either attract or repel insects to/from the target plant. When the neighboring plant is unpalatable to target plant herbivores, the predators are either repelled from the area or spillover occurs. Interestingly, when considering this effect using polyphagous insects, the results found were not significant, implying that plant species with narrower host ranges are a driving force (Barbosa *et al.* 2009). Next, the taxonomic similarity between a target plant and its neighbor may increase interactions leading to associational susceptibility. This is a logical conclusion because plants that are closely related are more likely to share herbivores.

Neighboring plants increase the likelihood of this herbivore detection and predation of target plants (Barbosa *et al.* 2009). The occurrence of associational susceptibility may be more impacted by relative abundance of neighboring plants than specific traits of neighboring plants. Therefore, a greater intensity of traits yields higher predation. However, this can be largely confounded by plant species biomass, meaning

that individual plant species vary greatly in size. As a result, plants that offer a greater resource attract greater herbivore predation.

Long-term detriments to target plants, like decreases in fitness or yield, experiencing associational susceptibility depend on the consistency and strength of this concentration as well as the variety of direct/indirect interactions between plants. Direct mechanisms imply the individual's own traits impact damage, whereas indirect mechanisms attribute damage to neighboring individuals. The former allows for higher resistance to evolve through heritable traits found within a given population; the latter does not. Instead, indirect mechanisms rely on the landscape to inform management strategies.

Ultimately, indirect interactions like associational susceptibility impact the overall fitness of the target plant, but because they depend on characteristics of the neighborhood and not heritable traits of the target plant, there can be no evolutionary response to the fitness consequences. Unfortunately, ecosystems lend themselves to large quantities of direct and indirect interactions making it difficult to predict the relative importance of any particular interaction, like associational susceptibility. However, as the detrimental effects of associational susceptibility accumulate, the fitness of the target plant species decreases. As a result, it is important to study a breadth of indirect associational effects within the complex environment of an individual target species. When limiting a study to a controlled environment, like a lab setting, important mechanisms contributing to associational effects may be disregarded.

Here, if we had just considered *L. planus* and *C. pitcheri* interactions, associational effects may have been overlooked.

Study System

Release of a non-native flower-head weevil, *Larinus planus*, as a means of controlling Canada thistle, *Cirsium arvense*, epitomizes a biological control failure. Originally released as biological control for one of the most serious perennial weeds of agriculture in the cooler temperate regions of the world, *C. arvense*, *L. planus* widely prefers Pitcher's thistle and is therefore contributing to the steady decline of population sizes (McClay, 1988). Seven species were released as biocontrol agents for Canada thistle; four of those were weevils, including *L. planus*. Interestingly, *L. planus* (previously *L. carlinae*) was evaluated twice as a potential biocontrol agent for Canada thistle. Its introduction was rejected in the 1960s due to its diet breadth in European host-specificity tests (Zwölfer *et al.* 1971). After *L. planus* was found in the northwestern United States, researchers continued to suggest redistribution of the weevil to areas



with Canada thistle (Wheeler *et al.* 1985). Using contemporary protocols to evaluate host specificity, McClay (1989) determined that the weevil was unlikely to form significant populations on native thistles.

Figure 1 Adult *L. planus*, on Canada thistle, photographer: David Cappaert

Sadly, McClay failed to consider the flower head difference between native and Canadian thistles in his study (Louda *et al.* 2003). This further supports Louda *et al.* claim that test environments are not sufficient to predict responses in a new environment.

L. planus is a seed-eating weevil from Eurasia that is thought to have entered the United States accidentally in the 1960s (Havens *et al.* 2012). Adult *L. planus* span 5-8 mm long, 3 mm wide and are grey-blue in color (Deneke *et al.* 2008). The defining characteristic of weevils, their elongated snout, is called the rostrum. The elytra typically have one thin patch of grey to white hairs. Older individuals may appear completely black due to loss of colored hairs. Female *L. planus* grow a rostrum that is curved such that it is approximately 16.8% of the total female body length (Havens *et al.* 2012).

Along the coast of Lake Michigan, *L. planus* is threatening the survival of an endangered dune thistle, *Cirsium pitcheri* (Pitcher's thistle). Federally threatened since 1998, *C.*

pitcheri has fallen victim to a non-native weevil, *L. planus*. (Havens *et al.* 2012). Weevils were first discovered in Whitefish Dunes State Park (WDSP, Door County, WI) in 2010.

Havens and her team found weevils in one-third of the flower heads examined. The following year, the researchers found *L. planus* in over half of the flower heads

surveyed.

Weevil predation is driven by reproduction. Females oviposit their eggs into adult *C. pitcheri* capitula and the larvae then consume the thistle's seeds, preventing that capitulum from reproducing. The process of finding a host plant is largely unclear but chemical stimuli appear to play an important role in the selection process (Volovnik, 2015). Location and accessibility of the host plant also impact weevil selection (Hakes *et*

al, unpublished data). WDSP offers different habitat conditions for Pitcher's thistle. Therefore, an individual plant's associations determine the likelihood of detection by predators, like *Larinus* (Barbosa *et al.* 2009).

Since larvae development occurs within a capitulum, selection of the 'womb' is crucial for offspring survival. Factors affecting oviposition are size of the capitulum, ripeness of the capitulum and the presence of oviposition holes on the capitulum (Volovnik, 2015). Typically, female weevils prefer laying eggs in larger flower heads, where the mean diameter of the capitula examined was 13.54 mm (Volovnik, 2015). Ripeness of the flower head is attributed with specific stages of capitula development, where the head is sufficiently mature but either totally or mostly closed (Volovnik, 2015). It is unknown if a female can determine larval infestation of a specific capitula since site selection usually takes no more than a minute (Volovnik, 2015).

After selection, oviposition occurs. This is the most arduous phase of the oviposition process. Females use their rostrum, to remove enough plant matter to reach the base of the capitulum. Longer and more pointed rostra are associated with the *Larinus* weevils that lay eggs in still closed flower heads (Brandl *et al.* 1989). When gnawing out the hole, the female is constantly moving her body in hopes of compacting the walls of the hole. The prepared hole ranges from 2 mm to 4 mm wide (Volovnik, 2015). The female then turns and detects the hole with the tip of her abdomen, ovipositing her egg into the hole. Some species of weevil use their long rostra to push the egg deeper into the hole (Gültekin, 2006). The behavior with which the hole is sealed varies among weevil species. Often, female *Larinus* cover the egg with a droplet

of dense substance released from the tip of the abdomen. This fecal cap soon dries as a solid, which protects the egg from desiccation and zoophages. Many weevils are also known to mark oviposition holes with short-lived special pheromones, rendering the host an “occupied plant” but no such data are available for *Larinus* weevils (Kozlowski *et al.* 1983). The brief duration of the cues seems to suggest that older larva have the opportunity to damage or eliminate later eggs. Larva usually span 8-9 mm in length and the body is strongly curved in a ‘C’ (Deneke *et al.* 2008). Development usually spans 23-54 days. During this time, adult weevils are not particularly active, seeking refuge in leaf litter or in soil, where they will eventually overwinter.

Weevil consumption of *C. pitcheri* seeds yields obviously damaging effects for population densities of the flowering dune plant. Pitcher’s thistle, *Cirsium pitcheri*, is a prickly and pollinating addition to the shorelines of the upper Great Lakes. The disturbance prone habitat of the dune makes it the perfect home for this thistle. *C. pitcheri* is a monocarpic perennial, with 4-8 year life span, that can be described in four stages: seedling, juveniles with at least one true leaf, vegetative plants, and reproductive adult with flower heads. It flowers and reproduces once, then dies.



Figure 2 *C. pitcheri* with terminal and secondary heads at stages three and zero, respectively. Whitefish Dune State Park, 2016, photographer: Hakes

Despite a rather unusual life history, *C. pitcheri* contributes to the biodiversity of the dune by acting as a source of pollen. Flowering occurs in late June, where one plant can produce 1-35 pink/creamy-white capitula (Havens *et al.* 2012). The dune thistle is a sexually reproductive plant, therefore individual flower heads are not self fertilizing. Despite this, inbreeding can still occur as a pollinator may visit two flower heads on the same plant. Once fertilized, seeds develop and, over time, grow tufts of pappus, which allows them to catch breeze when the flower head opens. These characteristics, sexually reproduction and wind dispersal, initially give the impression of populations with high genetic diversity. Despite this, due stochastic processes like drift or founder events, *C. pitcheri* have relatively small population sizes leading to less genetic diversity within populations (Gauthier *et al.* 2010). Similarly, gene flow may also be limited due to *C. pitcheri* seed weight, meaning that seeds typically fall within 4 m of parent plant.

Obviously, *L. planus* predation is not the only factor endangering this plant species. Herbivory and trampling from native mammals, like deer, and birds, like the

American goldfinch (*Cardeulis tristis*), threaten the survival of this thistle. Other insects, like spittlebugs, cause apical meristem damage. Climate change also is predicted to restrict the range of *C. pitcheri* over the next decades (Vitt *et al.* 2010). Lastly, increased inbreeding among *C. pitcheri* greatly contributes to low diversity between populations (Gauthier *et al.* 2010).

Despite the plethora of variables contributing to its decline, *C. pitcheri* conservation is a worthy endeavor. As one of the only flowering plants located on dune communities, Pitcher's thistle attracts pollinating insects and therefore increases the biodiversity of the area. A source of pollen for migrating Monarch butterflies or local bees, *C. pitcheri* is a crucial component of the larger ecosystem. Lastly, learning how to successfully combat the spread of *L. planus* could provide future management options for failed biological control attempts.

Research Objectives

We hope to determine if associational effects of neighboring plants, specifically beach grass, affect weevil predation on Pitcher's thistle. Using these findings, we would be able to discern whether *C. pitcheri*'s neighboring community has a stronger correlation to plant damage than its physical characteristics. Based on previous findings from Hakes and her team, *C. pitcheri* are easier to access when denser patches of vegetation surround them. By observing weevil behavior, we hope to shed light on their locomotive preferences.

Study site

Whitefish Dunes State Park (WDSP) is a state park located on the eastern shore of the Door Peninsula in Wisconsin. Approximately 230 of the 867 acres of the park are considered the Whitefish Dunes State Natural Area, implemented to protect rare species of plant, such as the dune thistle (*C. pitcheri*) from the public (Wisconsin DNR, 2014). Dry sand and constant disturbance makes WDSP largely inhospitable for many plant species. The primary dune, nearest to the lakeshore, is inhabited by the early-successional beach-specialists, marram grass (*Ammophila arenaria*), which spreads its tuberous **roots** just under the surface of the sand. WDSP also has areas of high elevation, which refer to the dune slopes with areas of sandy blowouts (Meunier, 2015). Although *C. pitcheri* can be found here, these slopes harbor the fewest plant species due to the unstable nature of the sand. Contrastingly, the peak of the dune—with an elevation of approximately 12 m—yields the greatest plant diversity due to the adjacent late successional mixed broadleaf-coniferous forest that characterizes Door County (Meunier, 2015).

Experimental design



Figure 3 Sample *C. pitcheri* used for observational *L. planus* studies

Next, we wanted to consider direct mechanisms, by testing the relationship between flower head size and weevil damage. Here, height, crown diameter, head—capitulum—diameter, number of heads, head stage, head position and number of exterior oviposition holes per head were cataloged. Height referred to the length of the plant from the ground to its tallest head. Crown diameter represents the horizontal width of the plant from its base and it is a proxy for plant size. Number of flower heads also acts as a proxy for plant size. Head diameter indicates flower size and was measured using a caliper. Flower heads smaller than 10 mm were not considered. We assessed internal damage by the presence of *L. planus* found inside dead *C. pitcheri* flower heads. In August 2016, final damage assessments were conducted for all 97 *C.*

Weevils were collected in the morning of each observational day. They were readily available on adult *C. pitcheri* flower heads. Each weevil collected was marked with a unique color pattern on its elytra using Craft Smart paint markers.

Prior to each ethogram, air temperature, average wind speed, maximum wind speed, humidity and weather conditions were noted using a Kestrel Instrument (Minneapolis, MN). Any previous weevils were removed from the target plant to not influence dispersal behavior. *L. planus* was released on the sand 35 cm from the target plant. Once released, weevil behavior and duration of that behavior was recorded until the weevil reached the target plant, died, or flew out of sight. Cataloged weevil behaviors included: stationary (ST), walking (WA), mating (MA), foraging (FO), playing dead (PD) or flying (FL). If the observer lost track of the individual for a period of time the behavior was marked as 'out of sight' (OS). Locations of weevil behaviors included: sand (SA), grass (GR), juvenile or non-target *C. pitcheri* (CP), debris (DB) or dead grass (DG). The former refers to plant matter that is horizontal, whereas the latter refers to grass matter that is vertical. Behavior or locations not included in the scope of the ethogram were noted as XS or XL respectively. We released weevils from 17 separate target plant locations and conducted 170 weevil observations. Ethograms ranged from a total of 4 seconds to 2 hours. In addition to quantifying the number of seconds a weevil spent doing each behavior and at each location, we standardized the proportional time each weevil spent in a behavior or location.



Figure 5 *L. planus* on *C. pitcheri* leaf (left) and on an immature capitulum (flower head) *C. pitcheri* (right). Each weevil was marked for identification during ethogram study. Whitefish Dune State Park, 2016, M. Montoya

Statistical analysis

Statistical analysis was used to consider the relationship between grass neighbor associations and weevil damage. For all analyses that yield *P* values, we used an alpha value of 0.05. We analyzed the frequency of weevils flying out of sight from various locations (sand, grass, debris, or *C. pitcheri*) using a chi-square test of goodness-of-fit. Our expected frequency was an even ratio (1:1:1). We used the same test to analyze the dependent variable horizontal and vertical substrate. We continued to use chi-square test of goodness-of-fit when examining the location prior to reaching the target plant, combining 'grass' and 'dead grass' results as well as omitting 'out of sight' results, as this behavior was the fault of the observer. In order to analyze how time spent engaging in behaviors differed at various locations we conducted a chi-square test of independence. Next, to assess normality, we analyzed the distributions of weevil location and abiotic conditions using a Shapiro-Wilk test. Data that did not meet the assumption of

normality so we used the Spearman's rank correlation coefficient to explore the correlation between abiotic factors and time spent on behaviors/at various locations. We performed all of these statistical analyses in PAST (Hammer *et al*, 2001) and Excel templates (McDonald, 2014).

The main objective of our study was to determine if neighboring grasses affect weevil behavior and dispersal to Pitcher's thistle. All weevils were released on the sand, 35 cm from one of 17 target thistles. Of the 170 *L. planus* ethograms conducted, 75 (44%) weevils reached a target Pitcher's thistle, 91 (54%) flew away (i.e. long-distance dispersal event) and 4 weevils (1.8%) died under observation (Fig. 6). All 4 of the dead individuals died while struggling to upright themselves on the sand, where they were initially released. Even though the weevils were upright at the time of release, many weevils tended to fall on their dorsal side while on the sand (Czaplinska, personal observation).

We were interested in whether the 91 weevils that flew away (long-distance dispersal event) and did not reach the target plant, differed in their location (grass, sand, or debris) immediately prior to flying away (Fig.7). When combining 'grass' and 'dead grass' findings (both vertical substrate), 44 (48.4%) weevils flew away when on grass. Next, 34 (37.4%) weevils flew away when on sand, 8 (8.8%) when on debris, 3 (3.3%) when on a juvenile *C. pitcheri* and 2 (2.2%) when out of sight and unaccounted for. The difference in frequency of weevils flying away from the sand, grass, debris or juvenile *C. pitcheri* is significant ($\chi^2=53.247$, 3 d.f, $P<0.001$). However, this result is driven by the comparatively rare locations of 'juvenile *C. pitcheri*' and 'out of sight.' When just comparing the frequency of weevils flying away from horizontal substrate at ground level (i.e. sand and debris) with vertical substrate (i.e. dead grass, live grass, and juvenile

C. pitcheri) there is no association between location and this behavior ($\chi^2 = 0.281$, 1 df, $P=0.298$)

Similarly, of the 75 weevils that successfully reached the target plant, we examined whether the frequency of weevils at each location of the 'last stop' before flying or walking onto their host plant varied (Fig. 8). When combining 'grass' and 'dead grass' location findings, an overwhelming 64 (85.3%) weevils reached the target plant by either flying or walking directly from the vertical structure of grass. Next, 5 (6.6%) weevils reached the target plant from sand and debris. Lastly, 1 (1.1%) weevil reached the target plant after initially being out of sight by the researcher, and was therefore removed from the chi-square analysis. Here, the difference in proportions is significant ($\chi^2=94.081$, 2 d.f, $P<0.001$).

Next, we examined the frequency of time weevils spent engaged in each behavior. Of those successful 75 weevils, time was overwhelmingly spent stationary. Of the 75298 seconds of weevil observations, *L. planus* spent a total 50,237 seconds stationary compared to a total 13,027 walking (Fig. 9). The frequency of time spent in each behavior was significantly different between locations ($\chi^2=20.560$, 12 d.f, $p<0.001$). Weevils tended to be stationary the longest on grass (26,020 sec) and also tended to walk more often on grass (5,037 sec) than on sand, or debris. Overall, *L. planus* was found to be most active in grass (38,567 sec) and second most active on sand (19,870 sec). Total time is lowest on *C. pitcheri* vegetation, which is a combination of activity on immature *C. pitcheri* and the target plant prior to reaching the flower head (6848 sec). This may be a reflection on how the study was conducted (as noted above, not every

thistle release site had juvenile *C. pitcheri* nearby). The proportion of time spent flying away was highest in sand environments (14.14% on sand, 2.23% on grass, 1.03% on debris, 1.08% on juvenile *C. pitcheri*).

The association between abiotic factors (air temperature, wind speed and humidity) and total/proportional time spent on grass, sand and debris location was examined (Table 1). The variable 'Total time' represents how long we observed an individual before it either died, flew away or reached the target thistle. In general, less total time dispersing was observed as air temperature increased ($r=-0.384$, $P < 0.001$) and more total time was spent at various locations as humidity increased ($r=0.321$, $P < 0.001$). Interestingly, this association differed when only considering the weevils that successfully reached the target plant (Table 4). Here, proportion of total time increased as air temperature increased ($r=0.272$, $P=0.019$) and proportion of total time decreased as humidity increased ($r=-0.287$, $P=0.013$). Next, a significant correlation was found between sand and the abiotic factors air temperature and humidity (Table 2). As humidity increased, time spent on the sand increased ($r=0.310$, $P < 0.001$). However, as air temperature increased, time spent on the sand decreased ($r=-0.336$, $P < 0.001$). This correlation was also found among the weevils that successfully reached the target plant (air temperature: $r=-0.330$, $P=0.004$, humidity: $r=0.306$, $P=0.008$, Table 3). An almost identical correlation was found between time spent on debris and the abiotic factors air temperature and humidity. Among all the weevils, total time spent on debris decreased as temperature increased and time spent on debris increased as humidity increased (air temperature: $r=-0.361$, $P < 0.001$, humidity: $r=0.389$, $P < 0.001$, Table 2). The same pattern

is seen when considering proportional time (air temperature: $r=-0.355$, $P<0.001$, humidity: $r=0.398$, $P<0.001$, Table 1). This was also reinforced among the weevils that successfully reached the target plant, where the proportion of time on debris decreased as air temperature increased and increased as humidity decreased (air temperature: $r=-0.417$, $P<0.001$, humidity: $r=0.436$, $P<0.001$, Table 4).

Finally, the effect of abiotic factors and total time spent doing various behaviors (stationary, walking, playing dead and out of sight) was examined. In general, time spent stationary or walking decreased as air temperature increased (stationary: $r= -0.349$, $P<0.001$, walking: $r= -0.376$, $P<0.001$, Table 5). Contrastingly, as humidity increased, time spent walking increased ($r= 0.344$, $P<0.001$, Table 5). These walking correlations were also found among the weevils that successfully reached the target plant (air temperature: $r= -0.288$, $P=0.012$, humidity: $r= 0.308$, $P=0.007$, Table 6). All behaviors, where the Spearman's rank coefficient was significant, decreased as temperature increased.

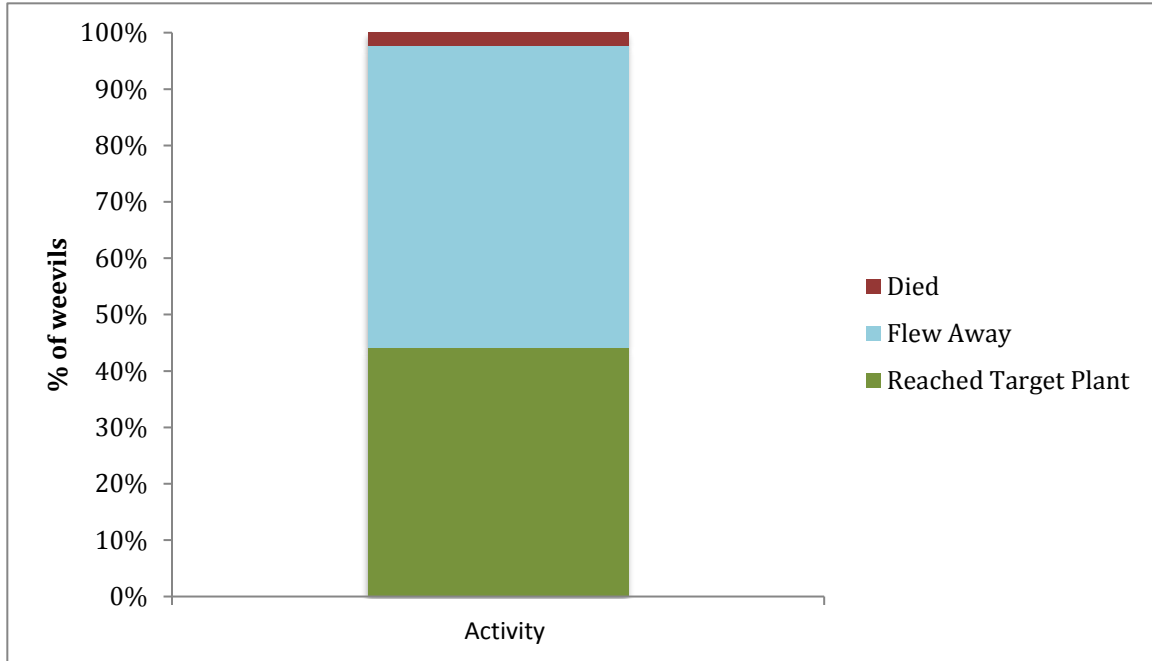


Figure 6 Total Ethogram Result—Frequency of *L. planus* that reached target thistle, flew away or died on the sand (N=170)

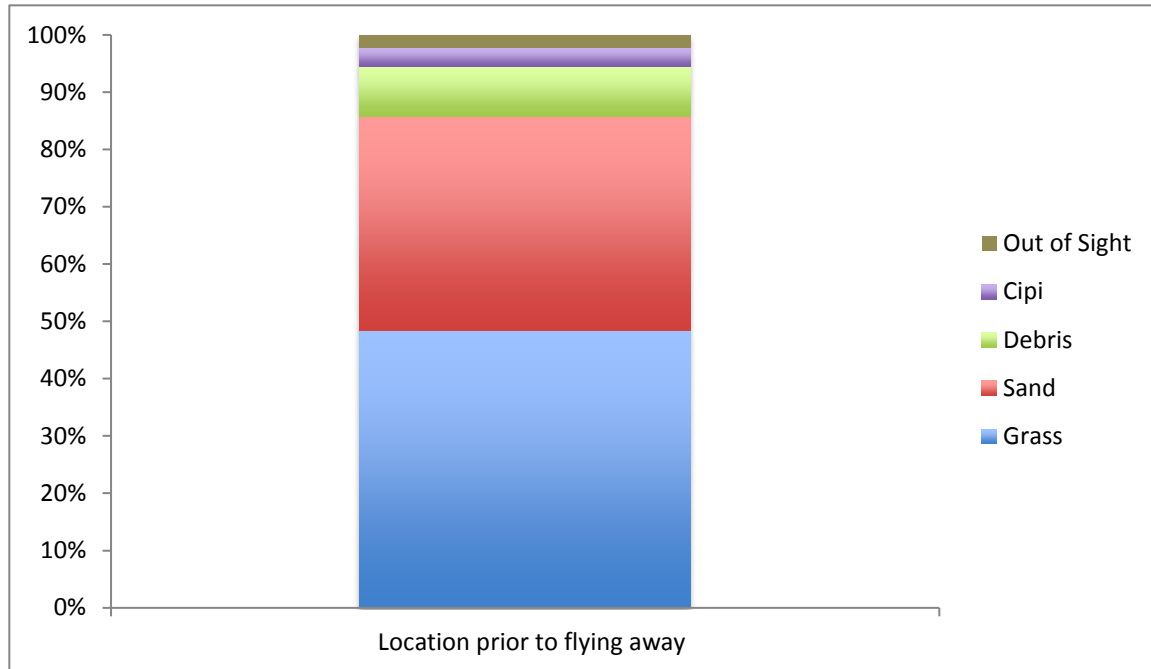


Figure 7 *L. planus* location prior to flying away—Frequency of *L. planus* that flew away from grass, sand, debris, juvenile *C. pitcherii* or were out of sight (N=91)

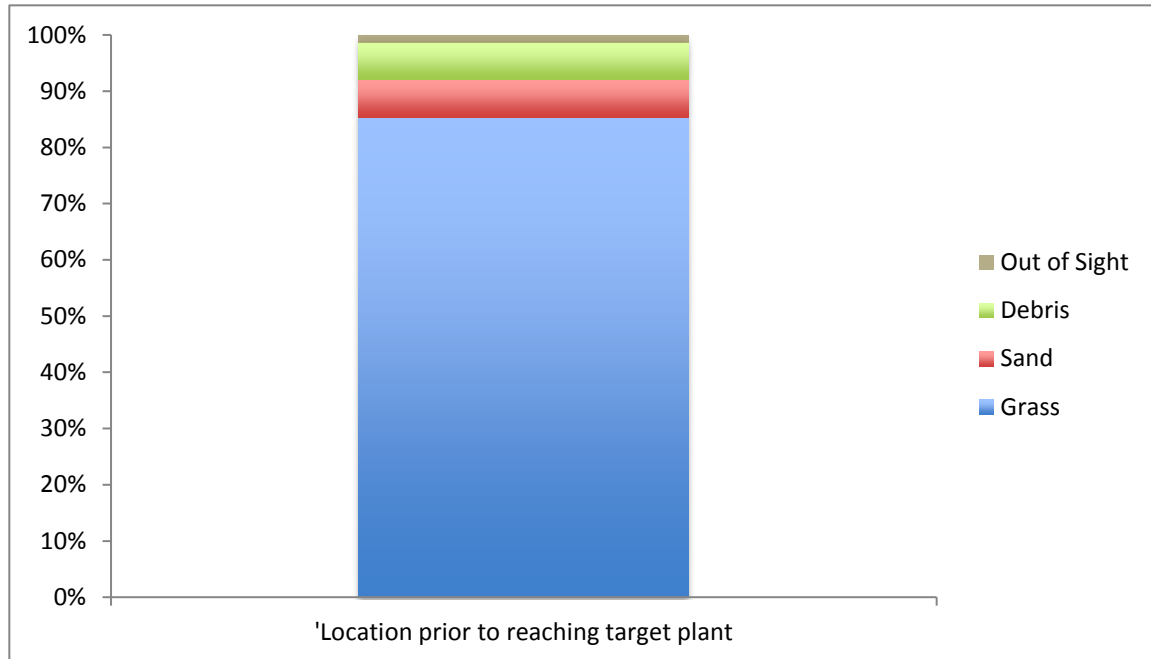


Figure 8 *L. planus* location prior to reaching target plant—Frequency of *L. planus* that reached target plant from grass, sand, debris or out of sight (N=75)

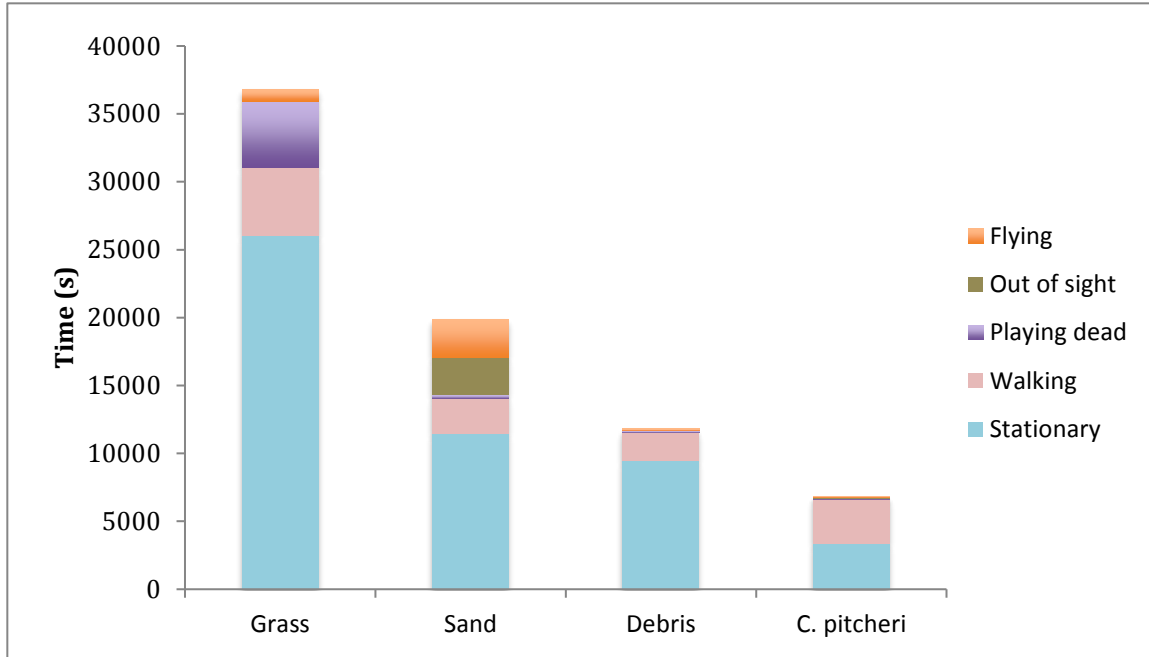


Figure 9 Behavior distributions across location—Time spent engaging in each behavior, which differed by location of the weevil. This analysis was restricted to *L. planus* that successfully reached target plant (N=75)

Table 1. Spearman's rank correlations between abiotic conditions and proportion of time (seconds) spent at various location, displaying rank correlation coefficient for all *L. planus* tested (N=170), where $p < 0.05^*$, $p < 0.01^{**}$ and $p < 0.001^{***}$

	Total	Prop. Grass	Prop. Sand	Prop. Dead Grass	Prop. Debris
Air temperature (°C)	-0.0384 ^{***}	-0.0905	0.0217	-0.162 [*]	-0.355 ^{***}
Avg. wind (m/s)	0.114	0.012	-0.131	0.256 ^{***}	0.054
Max. wind (m/s)	0.126	-0.009	-0.125	0.226 ^{**}	-0.016
Humidity (g/c ³)	0.321 ^{***}	0.071	0.017	0.093	0.399 ^{***}

Table 2. Spearman's rank correlation between abiotic conditions and total time (seconds) spent at various location, displaying rank correlation coefficient for all *L. planus* tested (N=170), where $p < 0.05^*$, $p < 0.01^{**}$ and $p < 0.001^{***}$

	Grass	Sand	Dead Grass	<i>C. pitcher</i>	Debris
Air temperature (°C)	-0.147	-0.336 ^{***}	-0.263 ^{***}	-0.137	-0.362 ^{***}
Avg. wind (m/s)	0.068	-0.008	0.231	-0.004 ^{***}	0.081
Max. wind (m/s)	0.055	0.0352	0.191 [*]	-0.008	0.012
Humidity (g/c ³)	0.109	0.310 ^{***}	0.199 ^{***}	0.168 [*]	0.388 ^{***}

Table 3. Spearman's rank correlation between abiotic conditions and time (seconds) spent at various location, displaying rank correlation coefficient for all *L. planus* tested (N=75), where $p < 0.05^*$, $p < 0.01^{**}$ and $p < 0.001^{***}$

	Grass	Sand	Dead Grass	<i>C. pitcheri</i>	Debris
Air temperature (°C)	0.109	-0.330**	-0.199	-0.198	0.401***
Avg. wind (m/s)	0.205	-0.072	0.234	-0.047	0.047
Max. wind (m/s)	0.239*	0.053	0.202	2.75E-05	0.037
Humidity (g/c ³)	-0.123	0.306**	0.158	0.229**	0.415***

Table 4. Spearman's rank correlation between abiotic conditions and proportion of time (seconds) spent at various locations, displaying rank correlation coefficient for all *L. planus* tested (N=75), where $p < 0.05^*$, $p < 0.01^{**}$ and $p < 0.001^{***}$

	Prop. Grass	Prop. Sand	Prop. Dead Grass	Prop. Debris	Prop. Total
Air temperature (°C)	0.234*	-0.072	-0.107	-0.417***	0.272*
Avg. wind (m/s)	0.181	-0.108	0.239*	0.038	0.169
Max. wind (m/s)	0.155	-0.096	0.161	-0.039	0.158
Humidity (g/c ³)	-0.225	0.121	0.068	0.436***	-0.287*

Table 5. Spearman's rank correlation between abiotic conditions and proportion of time (seconds) spent engaging in various behaviors, displaying rank correlation coefficient for all *L. planus* tested (N=170), where $p < 0.05^*$, $p < 0.01^{**}$ and $p < 0.001^{***}$

	Stationary	Walking	Playing dead	Out of sight	Flying
Air temperature (°C)	-0.3488***	-0.37629***	-0.072844	-0.23831**	-0.012206
Avg. wind (m/s)	0.050011	0.11268	0.048584	0.0038966	0.016657
Max. wind (m/s)	0.075526	0.099358	0.045238	0.0050225	-0.030313
Humidity (g/c ³)	0.31444***	0.34434***	0.079371	0.26357***	0.010318

Table 6. Spearman's rank correlation between abiotic conditions and proportion of time (seconds) spent engaging in various behaviors, displaying rank correlation coefficient for all *L. planus* tested (N=75), where $p < 0.05^*$, $p < 0.01^{**}$ and $p < 0.001^{***}$

	Stationary	Walking	Playing dead	Out of sight	Flying
Air temperature (°C)	-0.21497	-0.28781**	-0.17179	-0.27227*	0.14375
Avg. wind (m/s)	0.076841	0.051945	0.019729	-0.034543	0.052797
Max. wind (m/s)	0.20972	0.1362	0.13444	-0.010413	-0.073056
Humidity (g/c ³)	0.21585	0.30824**	0.12128	0.29121**	-0.14018

Our ethogram study provided insight into the dispersal and host-finding behavior of a non-native weevil that is attacking the rare, native Pitcher's thistle at WDSP. Overall, we found that marked weevils that reach their target host are overwhelmingly using grass neighbors to get to the thistle. In contrast, location (sand, debris, grass) had no effect on weevils that did not reach the host (i.e. flew away), but weevils were more likely to fly when on sand environments. Furthermore, our evidence supports that abiotic factors, such as temperature, affected the total time of dispersal and time spent engaging in certain behaviors and locations. Together, these results suggest that neighboring beach grass acts as a dispersal mediator for the non-native weevil, potentially explaining why thistles with grass neighbors suffer higher levels of weevil infestation than thistles in sandy locations. Knowing how weevil behavior is affected by thistle neighborhood and abiotic factors, we hope to better inform conservation efforts of *C. pitcheri* and management strategies to control insect pests.

Dispersal mediated effects

In general, neighboring grass facilitates *L. planus* dispersal. Weevils spent an overwhelming amount of their time on the neighboring grass. Weevils that reached the target plant spent most amount of time on the grass (48.8%). Moreover, 85.3% of the weevils that reached the target plant flew or walked directly from the grass to the

thistle (where grass was the 'last stop') before arriving. This suggests that target thistle hosts may be more susceptible to weevil damage in grassy environments because grasses are the substrate that weevils prefer to spend time on when looking for hosts. This finding offers a new and overlooked mechanism of associational effects.

Traditionally, associational susceptibility predicts that neighboring plants will serve as an alternative resource to herbivores (Agrawal, 2004; Barbosa *et al.* 2009). *L. planus*, however, does not feed on beach grass and instead feeds on fresh leaves of target host plants (Johnson *et al.*, 2008). Therefore, the grass is serving *L. planus* mainly—based on the results of this study—as a means of dispersal. Grass substrate might allow for the weevil to remain upright, which is a luxury that they are occasionally denied on the sand (Czaplińska, unpublished data). These findings imply that sand can negatively influence *L. planus* fitness. Sand was not as popular among the weevils that successfully reached the target plant, especially when compared to the weevils that did not reach the thistle (6.6% and 37.4%, respectively). Similarly, four weevils were observed dying on the sand after falling on their backs and struggling. We did not have a large enough sample to determine whether temperature was a determining factor in weevil mortality on sand, but we did observe these deaths on sunny, hot days (34.2°C, 20.2°C, 31.6°C and 30.1°C). However, if the grass was preferred solely because it is an alternative to sand, this is not supported by our observations. Debris, which offered a more stable horizontal substrate than sand, was not a preferred location for weevils. This may suggest that the vertical structure of grass dictates weevil location preference.

Other uses of the grass should also be considered, including: refuge from predators, camouflage, volatile chemical detection, or respite from stressful environments. A commonly observed *L. planus* defense mechanism was falling (Czaplinska, Hakes, Montoya-Paniagua; unpublished data). We observed the weevils' tendency to fall from *C. pitcheri* or grass when disturbed rather than fly away. Falling among these plants made it difficult for the observer to spot the weevil. This suggests that *L. planus* uses vertical structures, like the host plant and neighboring grass, as camouflage and protection. Sand does not appear to possess defense mechanisms for the weevil. Weevils, which are dark in color, are easy to find on the sand. Similarly, a weevil that 'trips' on the sand often struggles to return to an upright position. The four documented weevil deaths occurred on the sand after the individual had fallen onto its back. The lack of debris or vertical structures on some parts of the sand environment can make it difficult or impossible for the weevil to regain an upright position. This and other factors contribute to making the sand a stressful environment for *L. planus*. Our studies show that temperature negatively impacts time spent on the sand, suggesting that vertical structures may maintain cooler temperatures. Future studies could pursue distinguishing these preferences.

Grass may also be used as an aid for volatile chemical detection. Herbivorous insects typically rely on cues, visual or chemical, to find the target plants (Barbosa *et al* 2009). *L. planus* indisputable preference for *C. pitcheri* only at their reproductive stage suggests that flower heads can produce volatile chemical signals that allow for weevil detection. As a result, the vertical structure of grass might facilitate olfactory cue

detection by stationary weevils. *L. planus*' prominent geniculate, or elbow-like, antennae propose olfactory significance. How *C. pitcheri* volatile emission influences the behavior of the weevil can inform future management strategies. Plant volatiles have been found to enhance the 'appeal' of insect sex pheromones (Deng *et al*, 2004; Dickens, 1989; Light *et al* 1993). For example, green leaf alcohols have been found to attract cockchafer (*Melolontha* spp.) males to plants that females are already feeding on (Reinecke *et al*, 2002). A plant's chemical cues also cause some herbivorous insects to alter their production and release of sex pheromones (Reddy *et al*, 2004). These plant volatiles indicate a suitable food source for their progeny. For example, chemicals released by tomatoes—like ethylene and 3-methyl-butan-1-ol—induce sex pheromone production in females of the moth species *Helicoverpa* (Raina *et al*. 1992).

One alternative explanation for why weevils may prefer thistles with grass neighbors is that grasses and thistles may directly facilitate or compete in ways that affect thistle fitness. In this case, weevils may be drawn to the characteristics of the thistles themselves while choosing their host. Although we do not have evidence whether grass directly affects thistle fitness, our research findings allow us to minimize the role of thistle flower head traits being a direct mechanism affecting weevil damage. When considering the relationship between *C. pitcheri* flower head diameter and oviposition, the logistic regression revealed no significant relationship ($P=0.377$). This means that weevils are not searching for larger flower heads in which to oviposit. Previous research in our lab also failed to find relationships between thistle size and weevil damage (Hakes, unpublished data). Despite this, past studies have attributed *L.*

planus damage to Tracy's thistle's, *Cirsium undulatum*, physical characteristics (Louda *et al.* 2002; McClay, 1989). Here, most of the undamaged flower heads were the smaller heads on side branches. One reason why McClay observed different results than we did could be the large difference in flower head diameter between native *C. pitcheri*, which are larger, and Tracy's thistles, which are smaller (Louda *et al.* 2002). This may suggest that size of flower head matters to weevils up to a threshold, and then heads are large enough for weevil development. In our study, even the smallest *C. pitcheri* heads may be above that size threshold.

Our research did not consider the chemical effect of flowering heads on weevil dispersal behavior. Plant-chemical cues are found to affect oviposition behavior in herbivorous insects, where plant volatiles released from flowering plants play a vital role in guiding oviposition behavior (Reisenman *et al.* 2009). Plants can limit damage by changing flower-opening activity in response to damage (Hilker *et al.* 2011). For example, flowering and undamaged tobacco plants attract the Carolina sphinx moth (*Manduca sexta*) whereas tobacco plants that are damaged by moth larvae release less of the attractive volatile (Hilker *et al.*, 2011). Additionally, these damaged plants can reduce the percentage of flower opening during the night, when the moths are active. Observing a correlation between amount of flowering heads open and over all plant damage could be a useful avenue to consider for *C. pitcheri*. *L. planus* tend to oviposit in closed heads so *C. pitcheri* might be increasing the amount of flower heads open among damaged heads.

Abiotic factors

Air temperature and humidity appear to most impact weevil behavior. Our research generally reveals that the two abiotic factors are inversely related. Namely, higher air temperature and lower humidity cause less weevil activity. This combination has been proven potentially deadly to insect embryo (Little *et al.* 2007). Humidity concentrations within the leaf boundary layer can influence choice of oviposition site and affect egg development (Hilker *et al.*, 2011; Woods, 2010). The leaf boundary layer refers to the thin layer of air surrounding the surface of a leaf. Low humidity due to dry air may lead to dehydration of the egg. Our results support that increased humidity positively impacts weevil activity, which may result in more oviposition. Although we did not note oviposition behaviors in our ethogram studies, we did observe increases in stationary and walking behaviors as humidity increased, as well as increased time spent on *C. pitcheri*. Female weevils are stationary when they oviposit and weevils will often walk to each other on the thistle in order to mate (Czaplinska, unpublished data). As humidity decreases and temperature increases, a plant's increase in temperature causes the metabolic rate of the embryo to increase. This, in combination with increased water loss in the egg, is known to cause the stressed embryo to die. Embryonic activity was not within the scope of this study. However, host-finding behavior may be affected by what is best for weevil development.

Future Avenues

Our observational studies confirm the use of grass as a means of *L. planus* dispersal. This is particularly important, as the grass does not provide a food resource to the weevil, which is unique to associational relationships. Instead, the grass's vertical and temperate environment might provide a more stable environment for *L. planus* (Paniagua Montoya, unpublished results). Grass might also be more conducive as a means of detecting plant volatiles. Conducting behavioral studies testing olfaction would help assess its affect on weevil dispersal. A suggested model for these tests includes a dynamic Y-shaped olfactometer, where charcoal-cleansed air is pumped containing volatile sources (see methods Mayer *et al*, 2008; see methods Defagó *et al*, 2016). Through this, Mayer and his team recommend using sticky traps equipped with dispensers containing volatile implicates as a pest management strategy. Determining the relationship between weevil dispersal and volatile cues could be the key to saving *C. pitcheri* from extinction.

Although weevil physiology suggests an olfactory preference, visual cues should also be considered as a potential means of facilitating dispersal. Future observations could compare the dispersal abilities of functioning weevils to those that are visually impaired. Despite this, binding weevils may not be within the moral scope of all future researchers.

Our study also did not consider the many internal factors that affect weevil behavior. Food deprivation plays a crucial role in insect responses. Hunger can trigger an increase in sensitivity to host plant stimulus (Defagó *et al*, 2016; Bernays *et al*, 1994). Assessing starvation levels of *L. planus* and how this relates to their dispersal rate may

inform peak damage periods. Since *L. planus* is mostly active June-August, *C. pitcheri* might be damaged at a higher rate in the beginning of June, as new (and hungry) adults emerge. Insect age also affects sperm production. We attempted to distinguish males and females in this study, when collecting weevils that were mating. The male sweet potato weevil, *Cylas formicarius*, produces more sperm and is able to transfer more sperm to females successfully with age (Hiroyoshi *et al.* 2016). Older males were able to produce and transfer more sperm. Interestingly, mating failure still occurs and Kuriwada *et al.* (2013) reported that more than half female *C. formicarius* copulated with no sperm in their body, especially when females walked during mating. Although males do not make oviposition choices, they may still want to find flower heads in order to find a mate. Therefore, sperm production may also inform peak damage periods.

This study also did not address larval *L. planus* behavior. Although larvae remain inside the flower head, exploring their behavior would provide a complete understanding of behavior across the *L. planus* life cycle. Unfortunately, *L. planus* observational studies would be difficult to execute in the field and would have to be conducted in a lab setting. Dismissing the environmental complexes can result in an incomplete understanding.

Currently, *L. planus* continue to severely threaten the *C. pitcheri* species. By studying weevil behavior, we hope to impact future management strategies to salvage *C. pitcheri* populations at Whitefish Dunes State Park and all along the coast of Lake Michigan. Ecology has the unique purpose of creating an environment suitable for all.

The havoc *L. planus* wreaked on *C. pitcheri* emphasizes the delicacy of every ecosystem, where no variable is truly independent. Therefore, all manipulations must take into account the greater complexity at hand. Fortunately, as dynamic beings, we are able to weed out insufficient methods and conduct more rigorous tests that incorporate host specificity and indirect associational effects. With these tools at our disposal, it is our duty to try to preserve the balance of the environment.

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