Araceli Morales
August 15, 2013

The effect of apical meristem damage on the fitness of *Cirsium pitcheri*

Abstract

Plants are known to exhibit a compensatory response to natural herbivory and other sources of damage. This study was intended to verify whether damage to the apical of meristem of *Cirsium pitcheri* resulted in overcompensation. We addressed the following questions: 1) Does pollinator activity differ between undamaged (single-stemmed) and damaged (multi-stemmed) *C. pitcheri*? 2) Is there a difference in fitness between single- and multi-stemmed individuals after apical meristem injury? To assess these questions we marked single- and multi-stemmed thistles at Sturgeon Bay, MI and surveyed various measures of fitness including, flowering and non-flowering heads, seed production, and pollinator activity. There was a significant difference in the number of total heads and seeds produced per plant between single- and multi-stemmed individuals. These results indicate that multi-stemmed *C. pitcheri* were exhibiting overcompensation after apical meristem damage, and thus increased their relative fitness relative to single-stemmed plants.

Introduction

Herbivory typically has a negative effect on plant growth and fitness (Agrawal 2000); however, some plants have developed an adaptation to tolerate herbivory by exhibiting a compensatory response (Olejniczak 2011). Plants sometimes overcompensate by reproducing to their maximum level, which has proved beneficial to their overall fitness compared to the undamaged plants of the same population (Alison et al. 2007). Studies concerning the mechanisms of overcompensation have found that hormones, transferred through the saliva of herbivores during the feeding process, may induce plant growth as well (McNaughton 1981). Still, other studies suggest that hormone transfer may not play a role in overcompensation; instead, moderate damage to the apical meristem may be the primary reason behind compensatory response by most plants (Paige and Whitham 1987). Numerous studies also indicate that enhancement of plant reproduction is likely to continue in the later years of the plant following natural herbivory and other sources of damage (Olejniczak 2011).

Because pollinator activity is essential for seed production for most plants, pollinator attraction to flowers is important. Previous studies have showed that plants with a larger number of flowers that are open at any one time have higher pollinator visitation time (Ohashi and Yahara 1998). Thus, if plants overcompensate by producing more stems, and consequently produce more flowers, damage to the apical meristem can also increase pollinator activity, due to having a larger floral display. In addition, floral display size can also affect pollinator consistency (Ishii, 2006), increasing the number of flowers visited on a plant.

Cirsium pitcheri (Pitcher's thistle) is a monocarpic perennial species endemic to the shoreline sand dunes of Lakes Huron, Michigan, and Superior (D'Ullsse and Maun 1996, Hamzé and Jolls 2002). Populations of *C. pitcheri* are most prevalent in the sand dunes of Lake Michigan where the shoreline currents and climatic patterns create an ideal open sand habitat for the plant (Hamzé and Jolls 2002). As a disturbance-dependent species, it may grow 3-10 years, flowering once, then disperses its seeds and dies (D'Ullssee and Maun 1996). *C. pitcheri* reproduces sexually, and while self-pollination is possible, outcrossing is better for successful seed fertilization; therefore, the plant relies heavily on insect-pollinator activity for seed fertilization (Havens et. al 2012, D'Ullsse and Maun 1996). With no vegetative reproduction, it is crucial that *C. pitcheri* has a successful seed production and seedling establishment in order to preserve the population.

In 1988 this rare species was listed as federally threatened due to its declining habitat (Girdler and Radtke 2006). Current threats to *C. pitcheri* range from anthropogenic activities, including habitat destruction and shoreline recreational use, to invasive herbivores and plants as well as trampling from animals such as white-tailed deer (Havens et. al 2012). The most recent threat observed is *Larinus planus* (Eurasian weevil) which was introduced to control invasive musk thistles (*Cirsium arvense* and *Carduus nutans*; Havens et. al 2012). Other stress factors include sand burial, sand erosion, desiccating winds, and extreme sand surface temperatures (D'Ullsse and Maun 1996). Other sand dune plants experience the same stressors; however, as a monocarpic plant, *C. pitcheri* has a higher risk of reduced population growth because of its reproductive limitations (Hamzé and Jolls 2000). In response to meristem damage, C. *pitcheri* exhibits compensation for lost tissue, increased mortality, changes in resource allocation patterns, and changes in the probability of flowering (Phillips and Maun 1996). After moderate apical meristem injury, *C. pitcheri* overcompensates for lost tissue by producing multiple rosettes, resulting in multiple flowering stems (Phillips and Maun 1996).

The purpose of this experiment was to determine if *C. pitcheri* exhibits overcompensation after damage to the apical meristem. We addressed the following questions: 1) Does pollinator activity differ between single- and multi-stemmed individuals? 2) Is there a difference in fitness between damaged (multi-stemmed) and undamaged (single-stemmed) *C. pitcheri* as a result of apical meristem injury? Since overcompensation often results in an increased number of stems, flowers, and seeds (Agrawal 2000), we predicted that multi-stemmed *C. pitcheri* would have greater fitness than single-stemmed individuals. To test our prediction we marked single- and multi-stemmed plants and surveyed various aspects of flowering and non-flowering heads, seed-set, and pollinator activity.

Study Site and Methods

Samples were taken during July 16th through the 22nd of 2013 among the dune complexes located at Sturgeon Bay (T38N, R5W) in Wilderness State Park, Emmet County, MI. Sturgeon Bay has relatively young sand dunes, around 40-75yrs old, that provide a prime open sand habitat for *C. pitcheri* and other plants including Marram grass (*Amophila* sp.), Lake Huron tansy (*Tanacetum huronense*), and Houghton's goldenrod (*Solidago houghtonii*). Because Sturgeon Bay is west-facing, there is more protection against extreme winds; however, abiotic factors including wind, water, erosion, and extreme surface temperatures were still present.

We established a 50x50 meter plot, covering both a primary foredune and interdunal areas of the sand dune, and marked all mature *C. pitcheri* within the plot. We recorded the total number of heads per plant for both single- and multi-stemmed individuals and measured the diameter of heads likely to flower (>12mm) and those not likely to flower (<12mm) with Vernier calipers. For each head we recorded the flowering stage, ranging from zero (unopened heads), six (past flowering but not dispersed) to D (completely opened and dispersed heads). To compare seed production, we harvested heads prior to dispersal (stage six) and recorded the number of filled (fertilized) and unfilled (unfertilized) seeds. Because *C. pitcheri* is a protected species, we harvested only 13 heads, and we collected no more than two heads per plant.

We estimated seed production by multiplying the number of filled seeds times the number of heads >12mm per plant. In order to determine whether floral display mattered for potential-pollinators we observed each plant for 10 minutes and recorded visitation time for each insect-pollinator. Finally, we recorded neighboring flowering plants within one meter around *C. pitcheri*. We used a t-test to compare means of the following variables in single- and multi-stemmed individuals: number of heads >12mm (large), number of heads <12mm (small), total number of heads, proportion of small heads to total heads, number of total potential-pollinators, total visitation time of potential-pollinators, number of filled seeds per head >12mm, and number of filled seeds per plant. We used a Chi-square test to determine the difference in frequency of head stage per plant.

Results

To determine whether multi-stemmed *C. pitcheri* overcompensated after apical meristem damage we used 10 different measures of relative fitness. In the 50 x 50m plot we marked and sampled a total of 34 plants. 11 single- and 23 multi-stemmed individuals were in the plot. Most *C. pitcheri* were located in the back of the primary dune complex with occasional clusters of both single and multi-stemmed thistles concentrated in the back of the primary dune as well. The significant differences of the measurements between the single- and multi-stemmed individuals were summarized in Table 1.

Total visitation time of potential-pollinators was significantly higher among damaged than undamaged *C. pitcheri* (t-test, $p < 0.05$, table 1, figure 1). Total visitation time was 6.66 times greater in multi- than in single-stemmed plants, with a mean of 27.55sc ±16.43 and range of 179 seconds per flowering head in single- and a mean of 183.67sc ±51.34 with a range of 891seconds in multi-stemmed individuals. The significantly greater visitation time in multi-stemmed plants could be due to the unusually high visitation time of a Bombid and Coleoptera species that stayed in the flower for the entire ten minutes of observation. Because we did not differentiate whether pollinator visitation time from one flower to another we could not calculate visitation time per flowering head and only did so per plant. The number of potential-pollinators was significantly higher in multi- than that of the single-stemmed individuals (t-test, $p < 0.05$, table 1, figure 2). We found no significant difference in the number of potential-pollinators per flowering head (t-test, $p < 0.05$, table 1, figure 2).

Multi-stemmed individuals significantly produced more total heads per plant than single-stemmed plants (t-test, $p < 0.05$, table 1, figure 3). Multi-stemmed thistles had significantly more heads >12mm compared to the single stemmed plants (t-test, $p < 0.05$, table 1, figure 3). Similarly, the number of heads <12mm was significantly higher in the multi- than that of the single-stemmed individuals (t-test, $p < 0.05$, table 1, figure 3). However, the average ratio of small heads was not significantly different between the damaged and undamaged *C.pitcheri*, with a mean of 18.31% ± 5.93 for single- and 23.04% ± 4.03 for multi-stemmed plants (t-test, $p < 0.05$, table 1). Although there was no significant difference in the number of flowering heads (stages 2-5) between single- and multi-stemmed plants, multi-stemmed thistles showed an increasing trend toward being greater (t-test, $p < 0.05$, table 1, figure 3, flowering single- N= 6, multi-stemmed thistles N=17).

The number of filled seeds per head >12mm was not significantly different between single- and multi-stemmed individuals (t-test, $p < 0.05$, table 1, figure 4). However, multi-stemmed *C. pitcheri* produced significantly more seeds per plant than did single-stemmed thistles (t-test, $p<0.05$, table 1, figure 5). Single-stemmed plants produced heads >12mm that ranged from 13.4mm to 24mm with a mean diameter of 17.21mm ±0.31mm and, whereas the mean diameter of heads in multi-stemmed plants ranged from 12mm to 25.7mm with a mean of 17.49mm ±0.2mm. Chi-square test showed no significance difference in the stage of heads >12mm between single- and multi-stemmed individuals ($X^2 = 3.28$, d.f. =7, $p < 0.80$, figure 6).

Discussion

There was a significant difference in the fitness between single- and multi-stemmed *C. pitcheri*. These results concurred with our predictions and are supported by previous studies. Plants can compensate for apical meristem damage and relative reproductive fitness may increase (McNaughton 1983). Based on the frequency of damaged and undamaged plants, multi-stemmed individuals constitute for more than half of the population of *C. pitcheri* within the study site. Because there were no differences in the number of small heads and filled seeds produced per plant, damaged individuals experienced an increase in relative fitness over undamaged plants. Multi-stemmed plants produced more heads >12mm. These heads have a higher chance of flowering before the plant dies; therefore, potential-pollinator activity can also increase and seed fertilization is likely to occur during the flowering season. In addition, the total number of potential-pollinators was larger in multi-stemmed individuals that can possibly be due to the proximity of other flowering plants including other *C. pitcheri*. The larger flower patch in the area could have influenced potential-pollinator attraction. This can explain the significant larger total visitation time per plant found in multi-stemmed plants, but it can also be due to outliers, in particular two species of insect-pollinators, that pollinated the plant for more than 10 minutes.

Multi-stemmed thistles exhibited overcompensation by producing a larger number of total heads per plant. With equal amounts of resources present for both groups, multi-stemmed individuals that produced more heads should have produced fewer seeds per head (Olejniczak 2000). Instead, *C. pitcheri* fully compensated in terms of seed production per head, this is supported by previous studies where no difference in seeds per fruit were found in *Raphanus raphanistrum* and *Ipomopsis aggregata* after induced herbivory (Lehtila and Strauss 1999, Paige and Whitham 1987). Even though multi-stemmed plants also produced more heads <12mm, both single- and multi-stemmed *C. pitcheri* were producing the same percentage of small heads on a per plant basis. However, Loveless (1984 in Jolls and Lin 1996 unpubl.) found no significant difference in the number of overall heads in single- and multi-stemmed plants. This partially supports our results in terms of observed compensation in the number of small heads by damaged plants, but does not concur with our significant results in the total number of heads per plant in the two groups.

We might expect multi-stemmed plants to also invest less resources in each head since they had almost twice as many heads as single-stemmed thistles; however, our study does not reflect this since there was no difference in the flowering stages between the two *C. pitcheri* groups, indicating that resource allocation was the same for both single- and multi-stemmed thistles. This was also demonstrated by Olejnizak (2011) who found no difference in flowering time or phenology between clipped and control *Sedum maximum*. Furthermore, from the potential-pollinators' perspective there seems to be no difference in the floral display in single- and multi-stemmed groups since the number of potential-pollinator per flowering head did not differ. However, previous studies showed that pollinator visit duration increased with floral display size (Ishii 2006); therefore, this is one measure of fitness that should be tested again. Essentially, successful pollination was equal between the two plant groups because seed production per head was the same as well.

Since our study did not focus on the amount of damage to the aboveground biomass in plant individuals, we could not suggest whether moderate or high damage to *C. pitcheri* resulted overcompensation. However, *Ipomopsis aggregata* exhibited overcompensation after 95% removal in aboveground biomass; whereas *Gentianella campestris* overcompensated after 50-70% damage (Paige and Whitham 1987, Huhta et al. 2000). These findings support the fact that the mechanisms that can lead to overcompensation can be intrinsic (changes in physiology or plant development), extrinsic (local biotic and abiotic environmental factors) and even species dependent (McNaughton 1983). The possibility that the results found by Jolls and Lin (1996 unpubl.), where they observed no significant difference in the potential-reproductive output of *C. pitcheri* at Sturgeon Bay, was due to existing local environmental conditions at the time of study. Such conditions include competition with other plants, nutrient availability, and timing of hebivory or damage to the apical meristem (Maschinski and Whitham 1989). Another consideration is the samples size. In this 1996 study there was 20% multi-stemmed thistles, whereas our study consisted of 67% multi-stemmed individuals.

Since overcompensation has been a widely debated subject, it is critical to note that apical meristem damage can come from a variety of sources other than hebivory including fires, winter storms, trampling, etc. (Beslky et al. 1993). Beslky (1993) argued that plants do not necessarily show overcompensation when exhibiting increased plant growth. Recent studies, however, have used direct measures of fitness including, flower head production, fruits and seeds per plant to measure overcompensation (Lehtilla and Strauss 1999, Paige and Whitham 1987, Olejnizak 2000). Similarly, we observed improved fitness in multi-stemmed plants and thus they were overcompensating after damaged to the apical meristem.

For future studies, we suggest a longer study time to closely observe flowering rate, pollinator visitation time and the specific sources of damage to *C. pitcheri.* Recording the actual distance of other flowering neighbors from each *C. pitcheri* may provide more information on potential-pollinator relationship with floral display. Overall, it seems that damaged individuals benefited from berbivory and other sources of injury. In damaged thistles, we observed compensation in the percentage of small heads per plant and the number of viable seeds per head. In the end, damaged individuals, however, overcompensated in the number of total heads and viable seeds per plant; thus, multi-stemmed thistles had an increased reproductive success. Finally, it is hard to predict the future survival of *C. pitcheri*. With declining sand dune habitat, it is wise to further analyze the evidence of overcompensation exhibited by *Cirsium pitcheri* in order to properly conserve current and future populations of this plant.

Fitness Measures	t	d.f.	p-value
Total visitation time of potential-pollinators	2.896	26.133	0.004
Total number of potential-pollinators	2.888	31.535	0.0035
Number of potential-pollinators per flowering head	1.086	21	0.145
Number of heads	2.891	32	0.0035
Number of heads >12mm	2.973	32	0.003
Number of flowering heads (stages 2-5)	1.494	21	0.075
Number of heads <12mm	1.964	31.584	0.029
Percentage of heads <12mm	0.664	32	0.256
Diameter of heads >12mm	0.773	123.714	0.221
Filled seeds per head	0.846	11	0.416
Filled seeds per plant	4.454	29.713	0.000

Table 1. Summary of t-test values of 11 measures of fitness for *Cirsium pitcheri*, with significant difference at the 0.05 level.

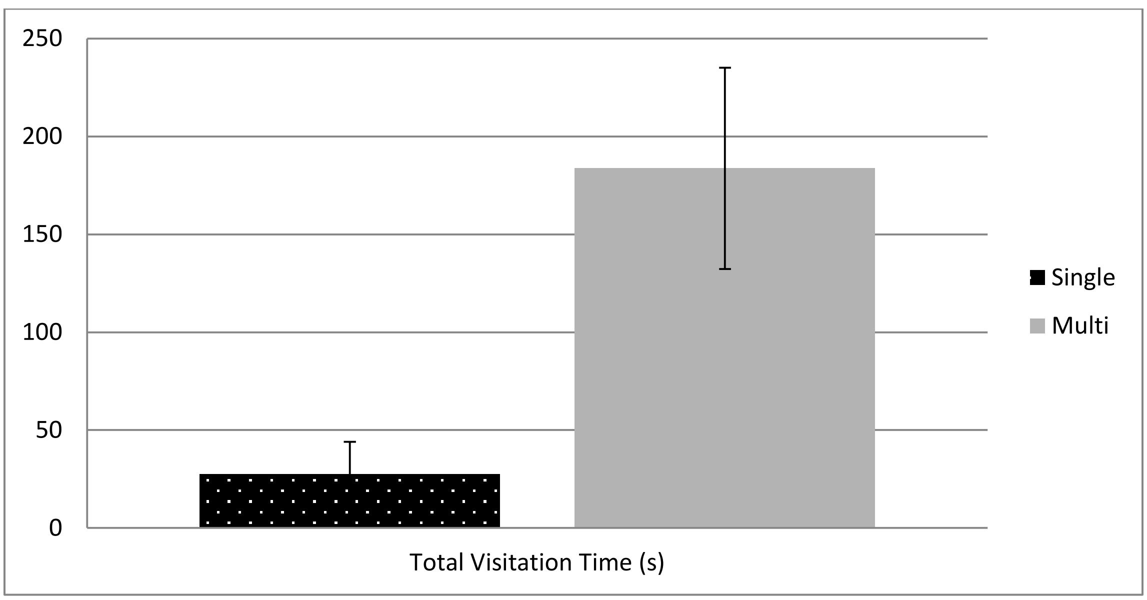

Figure 1. Differences in means of total visitation time per plant between single- and multi-stemmed *C. pitcheri*. Total visitation time was significant different in single- and multi-stemmed groups (t=2.896, d.f.=26.1, p<0.004).

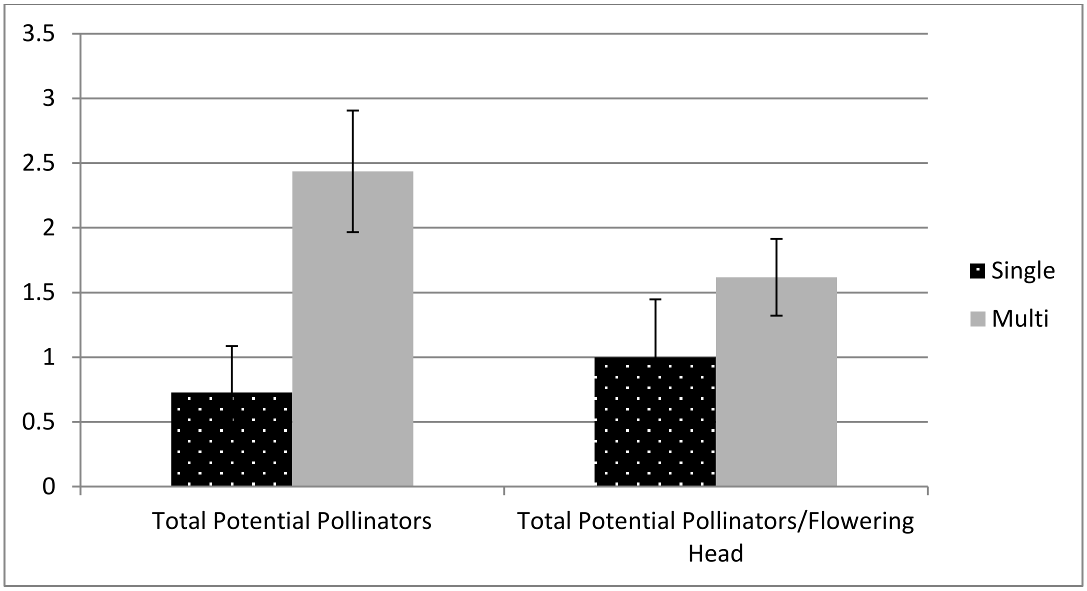

Figure 2. Differences in means of total potential-pollinator per plant and total potential-pollinator per flowering head between single and multi-stemmed *C. pitcheri*. Total potential-pollinators were significantly different between single- and multi-stemmed plants ($t = 2.88$, d.f = 31.5, $p < 0.0035$). Total potential-pollinators on a per flowering head basis was not significantly different between single- and multi-stemmed plants ($t=1.086$, d.f=21, $p<0.145$).

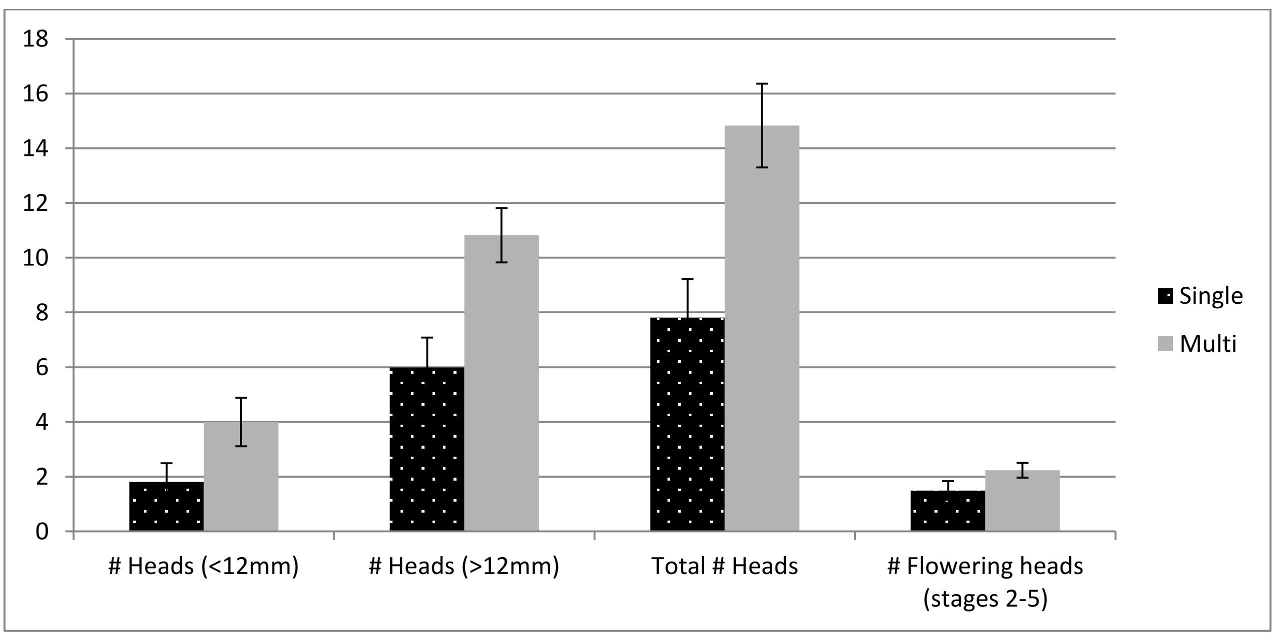

Figure 3. Differences in means of number of heads <12mm, number of heads >12mm, total number of heads, and number of flowering heads (stages 2-5) per between single and multi-stemmed plants. There was a significant difference in the number of heads <12mm ($t=1.964$, d.f.=31.6, $p<0.029$), in the number of heads >12mm ($t=2.973$, d.f.=32, $p<0.003$), and the total number of heads ($t=2.891$, d.f=32, $p<0.0035$). There was no significant difference in the number of flowering heads (stages 2-5, $t=1.494$, d.f.=21, $p<0.075$).

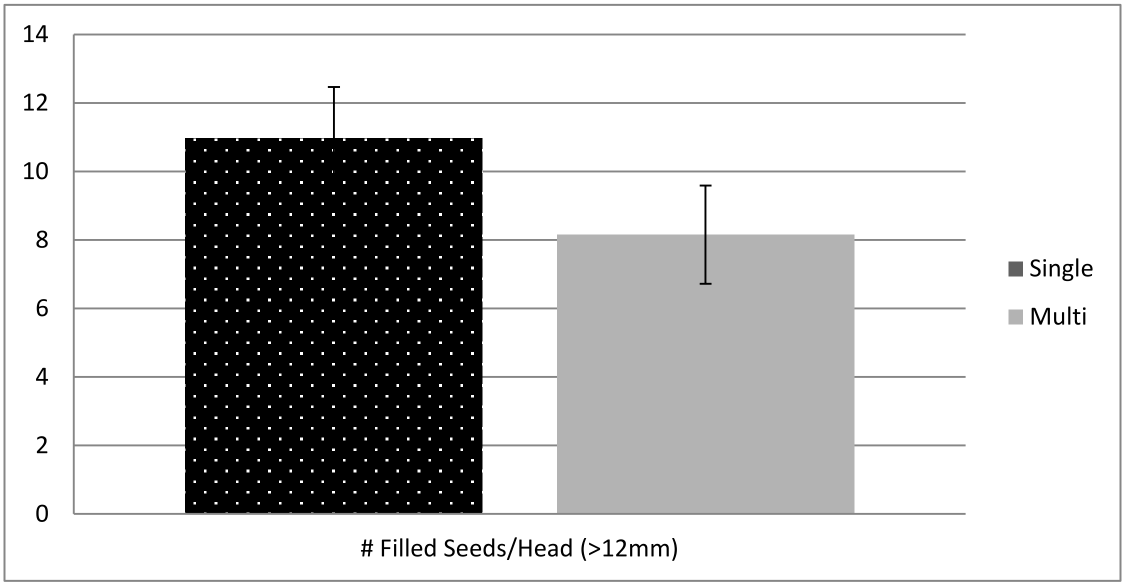

Figure 4. Differences in means of the number of filled seeds per head >12mm in *C.pitcheri*. There was no significant difference in the number of filled seeds per head >12mm between single- and multi-stemmed groups (t=1.209, d.f=32, p<0.118).

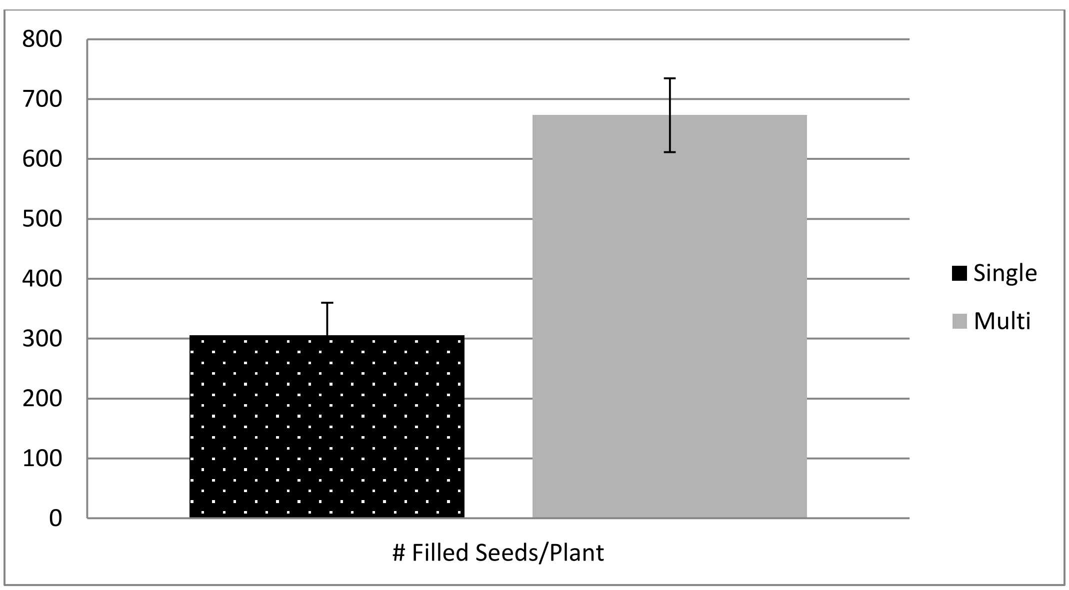

Figure 5. Differences in means of the number of filled seeds per plant in single- and multi-stemmed *C. pitcheri*. There was a significant greater number of filled seeds per plant in multi- than that of single-stemmed plants (t=4.454, d.f.=29.7, p<0.000).

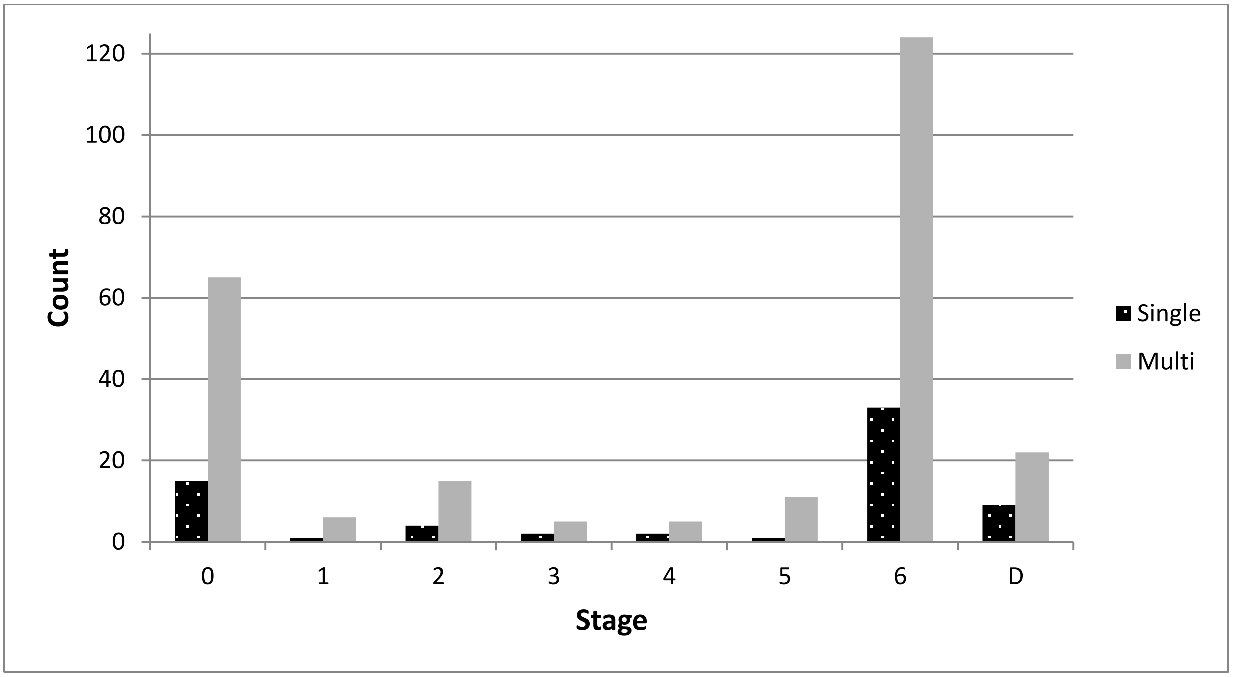

Figure 6. Differences in frequency of heads in stages 0-6 in single- and multi-stemmed *C. pitcheri*. There was no significant difference in the stage of heads >12mm between single- and multi-stemmed individuals ($X^2 = 3.28$, d.f. =7, $p < 0.80$).

Araceli Morales
August 15, 2013

Acknowledgements

We are grateful to our General Ecology professors, Kristen Uthus and Robert Pillsbury. They were really supportive in every step of the project and their suggestions and advice is what made this project possible. We also want to thank Claudia Jolls for allowing us to conduct our study in her research location and for her help with data collection. Her knowledge of *Cirsium pitcheri* was extremely helpful during the writing process.

Literature Cited

Agrawal, A. A. 2000. Overcompensation of plants in response to herbivory and the by-product benefits of mutualism. Trends in Plant Science 5: 309-313.

Alison, B. K., M. V. Price, and N. M. Waser. 2007. Life-history consequences of vegetative damage in scarlet gilia, a monocarpic plant. Oikos 116: 975-985.

Belsky, A. J., W. P. Carson, C. L. Jensen. 1993. Overcompensation by plants: herbivore optimization or red herring? Evolutionary Ecology 7: 109-201.

D'Ullsse, A. and M. A. Maun. 1996. Population ecology of *Cirsium pitcheri* on Lake Huron sand dunes: II. survivorship of plants. Canadian Journal of Botany 74: 1701-1707.

Girdler, B. E. and T. A. Radtke. 2006. Conservation implications of individual scale spatial pattern in the threatened dune thistle, *Cirsium pitcheri*. The American Midland Naturalist 156: 213-228.

Hamezé, S. I. and C. L. Jolls. 2000. Germination ecology of a federally threatened endemic thistle, *Cirsium pitcheri*, of the Great Lakes. The American Midland Naturalist 143: 141-153.

Havens, K., C. L. Jolls, J. E. Marik, P. Vitt, K. A. McEachern, and D. Kind. 2012. Effects of non-native biocontrol weevil, *Larinus planus*, and other emerging threats on populations of the federally threatened Pitcher's thistle, *Cirsium pitcheri*. Biological Conservation 155: 202-211.

Huhta, A., T. Lennartsson, J. Toumi, P. Rautio, and K. Laine. 2000. Tolerance of *Gentianella campestris* in relation to damage intensity: an interplay between apical dominance and hebivory. Evolutionary Ecology 14: 373-392.

Ishii, H. S. 2006. Floral display size influences plant choice by bumble bees. British Ecological Society 20: 233-238.

Jolls, C. L. and T. Lins. 2011. Apical damage and reproductive output of a Great Lakes endemic, *Cirsium pitcheri*. Unpublished data.

Lehtila, K. and S. Y. Strauss. 1999. Effects of foliar on male and female reproductive traits of wild radish, *Raphanus raphanistrum*. Ecology 80: 116-124.

Maschinski, J. and T. G. Whitham. 1989. The continuum of plant response to hebivory: the influence of plant association, nutrient availability, and timing. The American Naturalist 134: 1-19.

McNaughton, S.J. 1983. Compensatory plant growth as a response to herbivory. Oikos 40: 329-336.

Ohashi, K. and T. Yahara. 1998. Effects of variation in flower number on pollinators visits in *Cirsium purpuratum* (Asteraceae). American Journal of Botany 85: 219-224.

Olejniczak, P. 2011. Overcompensation in response to simulated herbivory in the perennial herb, *Sedum maximum*. Plant Ecology 212: 1927-1935.

Paige, K. N. and T. G. Whitham. 1987. Overcompensation in response to mammalian herbivory: the advantage of being eaten. The American Society of Naturalists 129: 407-416.

Phillips, T. and M. A. Maun. 1996. Population ecology of *Cirsium pitcheri* on Lake Huron sand dunes: I. impact of white-tailed deer. Canadian Journal of Botany 74: 1439-1444.