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The Effects of Apical Meristem Damage on the Fitness of *Cirsium pitcheri*

Abstract

This purpose of this study was to determine whether the Great Lakes endemic *Cirsium pitcheri*, Pitcher's thistle, exhibits overcompensation following damage to its apical meristem. We addressed the following questions: (1) Does pollination frequency differ between single-stemmed and multi-stemmed individuals? (2) Is there a difference in fitness between single-stemmed and multi-stemmed individuals? To assess these questions, we observed potential pollinator rates, head counts and diameters, and viable seed sets on both undamaged and damaged Pitcher's thistles. Total potential pollinator visits and pollination time per flowering head were significantly greater on multi-stemmed thistles. Total head count was significantly greater on multi-stems without a significant increase in the percentage of heads <12mm that will not flower or contribute to reproduction. Viable seed sets per head were not significantly different in multi-stem heads, resulting in a significantly greater number of viable seeds per multi-stemmed plant. Our data suggest that sublethal damage to the apical meristem of *C. pitcheri* from herbivory or trampling causes overcompensation, therefore having a positive effect on pollination rates and overall fitness.

Introduction

Damage from herbivores, trampling, abiotic factors, and anthropogenic disturbance can be some of the major influences on a plant's fitness. Damage can reduce fitness and be detrimental to the affected plant (Louda and Potvin 1995; Freeman *et al.* 2003), or plants can compensate for sublethal levels of damage so that there is no net change in the plant's fitness (Rand 2004; Strauss *et al.* 2001). However, a third potential outcome for a damaged plant is that of overcompensation, demonstrated by an increase in a plant's fitness following herbivory, trampling, or other damage (Paige & Whitham1987; Sanchez and Lasker 2004; Alward and Joern 1993). Damage to a plant's apical meristem can activate the growth of additional stems, leading to compensation and sometimes overcompensation to the damage received (McNaughton 1983).

Studies have shown that plants that have larger floral displays experience an increase in pollinator arrival rates (Eckhart 1991; Huang *et al.* 2006; Knight 2003; Ishii 2006). Additionally, pollination is often shown to be the limiting factor in reproductive success (Moody-Weis & Heywood 2001; Knight 2003). Hence, a plant that exhibits overcompensation by producing more flowers should attract more pollinators and ultimately, positively affect reproductive success and population viability.

Cirsium pitcheri, Pitcher's thistle, is a federally threatened monocarpic perennial endemic to the shoreline dunes of Lakes Michigan, Superior, and Huron, and is often subject to damage. The species prefers open, windblown sand dunes or low, open beach ridges (USFWS 2002) and is primarily found in foredune habitats with 70% open sand (McEachern et al. 1993). Pitcher's thistle requires four to eight years to mature, and when the individual is ready to reproduce, one single flowering stem emerges from the rosette. If damaged, the thistle is released from apical dominance, and produces multiple flowering stems instead. *C. pitcheri* is insect pollinated, predominantly by bees, but also by butterflies, skippers, flies, wasps, and some types of beetles (Keddy and Keddy 1984). The plant flowers once between June and September and then dies. Since the thistle does not reproduce vegetatively and because populations are being reduced by anthropogenic impact and deer grazing throughout much of its range (Phillips and Maun 1996), successful flowering and seed production is critical for population persistence.

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To better understand the influence of apical meristem damage on thistle fitness, we conducted a study to determine if *C. pitcheri* in the Sturgeon Bay sand dunes of Northern Lower Michigan exhibit overcompensation. We addressed the following questions: (1) Does pollination frequency differ between single-stemmed and multi-stemmed individuals? (2) Is there difference in fitness between single-stemmed and multi-stemmed individuals? Because *C. pitcheri* responds to apical meristem damage by producing multiple stems, we predicted damaged, multi-stemmed individuals would produce more flowering heads, attract more pollinators, and produce more viable seeds than undamaged single-stemmed individuals and, consequently, exhibit overcompensation. These questions were examined by comparing flowering head, seed, and pollination characteristics in single- and multi-stemmed Pitcher's thistles.

Methods

Data were collected at Sturgeon Bay in Wilderness State Park, Emmet County, in Northern Lower Michigan on July 16, 21, and 22, 2013. Sampled dune ridges ranged from approximately 40-75 years old (Lichter 1998). The area consisted of mostly bare sand with occasional surrounding vegetation including *Hypericum perforatum* (St. John's wort), *Ammophila* (marram grass), *Tanacetum huronense* (Lake Huron tansy), and *Solidago houghtonii* (Houghton's goldenrod).

Flowering-aged thistles were flagged within a 50m x 50m plot in an area encompassing primary foredune and posterior dune habitat. To observe differences in pollination frequency, each marked plant with open flowers was observed for 10 minutes to record potential pollinators and their time spent on the flowers. If a pollinator moved quickly from one flower to another within the same plant, the timing continued. It was also noted if a thistle had a flowering neighbor within a meter of it, including another flowering Pitcher's thistle.

To measure fitness, we surveyed each mature thistle and recorded whether it was a single- or multi-stemmed plant, the number of heads per plant, and the diameter of all heads.

Because probability of flowering is size-dependent in *C. pitcheri* (Jolls & Lin, unpubl. data), we distinguished between heads that would most likely not flower (<12mm) and those that would (>12mm.) All heads >12mm were classified by stage, ranging from zero to six depending on maturity, where zero was a completely closed head, and a six was a head that had flowered but not yet dispersed its seeds. Heads were classified as 'D' if the head was open and had already dispersed its seeds. To determine if there was a difference in viable seed production, 13 stage six heads were collected from both single- and multi-stemmed thistles. The diameter of each head was measured, and the number of filled (fertilized), unfilled (unfertilized), and total seeds was recorded. To calculate an average number of viable seeds per plant for single-stems and for multi-stems, we multiplied the average number of filled seeds for a particular stem type by the number of heads >12mm on each plant.

Independent sample, one-tailed t-tests were used to compare means between single- and multi-stemmed individuals for the following measures: the number of potential pollinators per plant, the potential pollinator visitation time, the total visitation time per flowering head, the number of heads >12mm, the number of heads <12mm, the number of heads per plant, the percentage of heads <12mm, the proportion of filled seeds to total ovules, the number of filled seeds per heads >12mm, and the number of filled seeds per plant. A p-value of less than or equal to 0.10 was used to determine the significance of Levene's Test when checking for equality of variances, and a p-value of less than or equal to 0.05 to determine the significance of the t-tests. A chi-square test was used to determine if there was a significant difference between the head stages of single- versus multi-stemmed thistles.

Results

A total of 34 mature, flowering-aged thistles were surveyed, of which 11 were single-stemmed thistles and 23 were multi-stemmed. Six of the 11 single-stems and 17 of the 23 multi-stems had flowers at the time of sampling. The mean total visitation time of potential pollinators and the mean total number of potential pollinators were significantly greater for multi-stems than single-stems (Table 1, Figs. 1&2). On single-stemmed plants, the average total visitation time was 27.5s, and 183.7s on multi-stems. The ranges of 179s and 891s, respectively, are large because beetles tended to stay on a flower for longer durations that bumble bees. Average number of pollinators per plant was 0.74 pollinators for single-stems and 2.4 pollinators for multi-stems. We did not find a significant difference in the average number of pollinators per flowering head, defined as flowers with stage two to five heads (Table 1, Fig. 1).

The mean number of heads >12mm, the mean number of heads <12mm, and the mean total number of heads were significantly greater on multi-stemmed individuals than on single-stemmed individuals (Table 1, Fig. 3). There was not a significant difference between the mean number of flowering heads, the mean percentage of heads <12mm, or the mean diameter of heads between single- and multi-stemmed thistles (Table 1, Fig. 3). For single-stems, the average number of heads >12mm per plant, the average number of heads <12mm per plant, and the average total heads per plant were 6, 1.8, and 7.8, respectively. For multi-stems, the averages were nearly double at 10.8, 4, and 14.8. The average number of flowering heads per plant was 1.5 for single-stems and 2.2 for multi-stems. There was not a significant difference between the flowering stages of single-stemmed and multi-stemmed Pitcher's thistles (X^2=3.28, df=7, p~.80; Fig. 6).

There was not a significant difference between the mean number of filled seeds per head >12mm on single- and multi-stemmed individuals, with averages of 50.9 and 62.2, respectively (Table 1, Fig. 4). The mean number of filled seeds per plant was greater for multi-stemmed individuals (Table 1, Fig. 5), with averages of 305.16 seeds per single-stemmed plant and more than twice as many seeds at 673.1 seeds per multi-stemmed plant, with ranges of 559.5 and 932.6 seeds, respectively.

Discussion

Results from this study demonstrate that the production of multiple stems due to apical meristem damage does cause an increase in pollinator activity. Total potential pollinators and visitation time were greater on multi-stemmed plants. Because the number of pollinators per flowering head was not significantly different between stem types, floral display size may not have affected which plant potential pollinators chose. Regardless, because multi-stemmed plants had more pollinators and greater total visitation time, the less likely it is that reproductive success of multi-stemmed *C. pitcheri* could be limited by pollination.

Data also show that multi-stemmed *C. pitcheri* do produce more total heads, on average, than single-stemmed *C. pitcheri.* Even though multi-stems produce a greater number of small heads per plant than single-stems, they do not produce a greater percentage of small heads, suggesting that damaged plants do allocate resources to produce a disproportionate amount of heads that will never flower or contribute to reproductive success, further supporting our prediction of overcompensation. Although the number of flowering heads on multi-stems was not significantly greater than on single-stems, the data show a clear trend toward being greater.

Results also show that multi-stemmed *C. pitcheri* do not over-allocate resources producing heads with fewer seeds than single-stemmed Pitcher's thistles. Furthermore, because the number of filled seeds per head does not differ significantly by stem-type and because multi-stems produce more viable heads on average, we saw that multi-stemmed Pitcher's thistles produce significantly more filled seeds per plant. The fact that more viable seeds are produced per plant supports our hypothesis that Pitcher's thistle does experience an increase in fitness and hence, exhibit overcompensation. Due to the threatened status of *C. pitcheri*, we were not able to collect a larger sample size of heads for seed count, but results may have been strengthened with a larger sample size of heads.

Our data show that *C. pitcheri* do exhibit overcompensation in terms of seed production when damage to the apical meristem results in a multi-stemmed plant, a result that disagrees with a previous study finding no evidence of overcompensation in Pitcher's thistles (Jolls & Lin, unpubl. data). This difference in recovery could be due to multiple factors, including pollination limitation, nutrient availability, and intensity of damage. Additionally, the sample of the previous study included only 20% reproductive multi-stemmed individuals, whereas our sample included 67% reproductive multi-stemmed individuals.

C. pitcheri reproduces only once in its lifetime so that the quantification of fitness is simplified, allowing overcompensation to be more clearly identified than in some other plants. Additionally, overcompensation is often environmentally-dependent (Agrawal 2000). A meta-analysis showed that dicots recovered significantly better in low resource conditions (Hawkes & Sullivan 2001). When such overcompensation is exhibited, it is often questioned why plants do not always reproduce optimally. The simple explanation is that a fully exposed plant may lose 100% of their production if fully developed when attacked by herbivores, suggesting that initial herbivory provides cues about the low risk of future herbivory (Agrawal 2000), demonstrated by *Ipomposis aggregata* (Paige 1999) and *Gentianella campestris* (Lennartsson *et al.* 1998) holding back the majority of their reproductive resources until after the initial and most intense herbivory. *C. pitcheri*, grazed by deer, do not quite present the same scenario in that the deer are not selective and will eat both juvenile and flowering thistles (Phillips & Maun 1996). This assumes the herbivores will not return to the same plant later on, or preferentially feed on the larger, multi-stemmed plants, a claim we cannot be sure of.

In light of the flaws in attributing our results solely to herbivory by deer, other sources of damage should be considered as the cause of the observed increased fitness. Plants experience damage from a wide variety of sources other than mammalian herbivory, including insect feeding on the bud of the rosette, trampling from deer hooves, or abiotic factors such as sand cover, wind, freezing, and fire. In fact, it is sometimes argued that rapid biomass regrowth is more like to have evolved as a strategy to reduce the negative impacts not only of herbivory, but of all types of damage (Belsky *et al.* 1993). In order for there to be overcompensation, there must be biotic damage early in the life cycle, saving of resources, and then reallocation of the saved resources into an increase in reproductively viable biomass. Further research should be conducted to determine the most dominant cause of apical meristem damage in *C. pitcheri*.

Our results showed that multi-stemmed Pitcher's thistles produce more viable heads, attract more pollinators per flowering head, and produce more filled seeds per plant than their single-stemmed counterparts, hence exhibiting the increase in fitness that defines overcompensation. Our study, along with others showing similar results (Paige & Whitham 1987; Sanchez and Lasker 2004; Alward and Joern 1993), support the argument that damage to the apical meristem by herbivory can benefit plants and enhance their fitness. Because hard evidence is limited to a few systems, further studies should be conducted to examine what plant characteristics, nutrient-availability, and environmental conditions are required for overcompensation to occur. Based on our findings, and for conservation purposes, a better understanding of overcompensation in *C. pitcheri* may prove to be very beneficial.

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	t	df	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 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: All independent samples one-tailed t-tests performed on the *Cirsium pitcheri* data from Sturgeon Bay in Northern Michigan. Levene's Test with a power of 0.10 was used to determine equality of variances.

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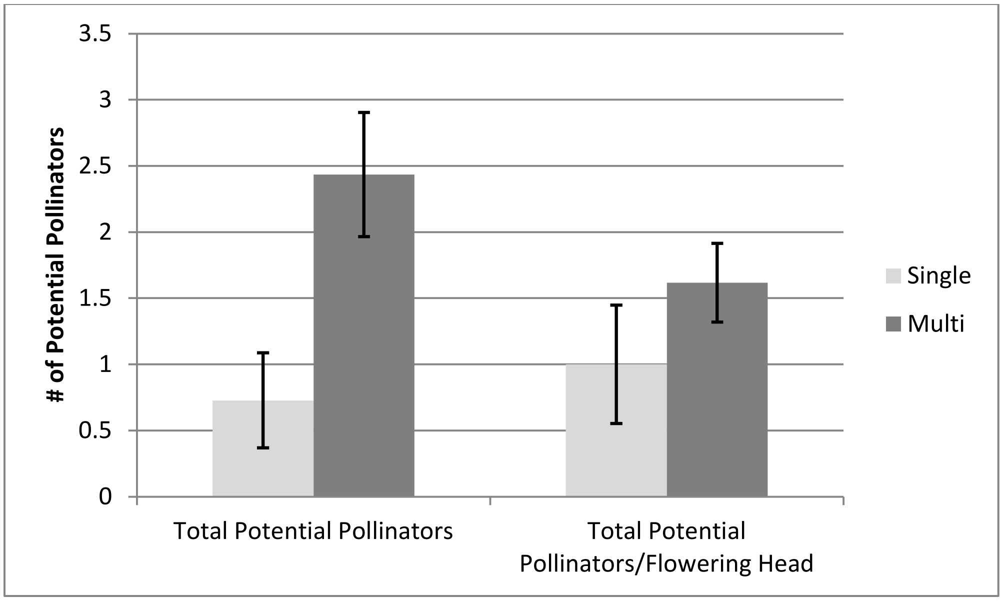

Figure 1: Average total potential pollinators and total potential pollinators per flowering head for single- and multi-stemmed *Cirsium pitcheri* of Sturgeon Bay in Northern Michigan. Bars represent the mean ± standard error.

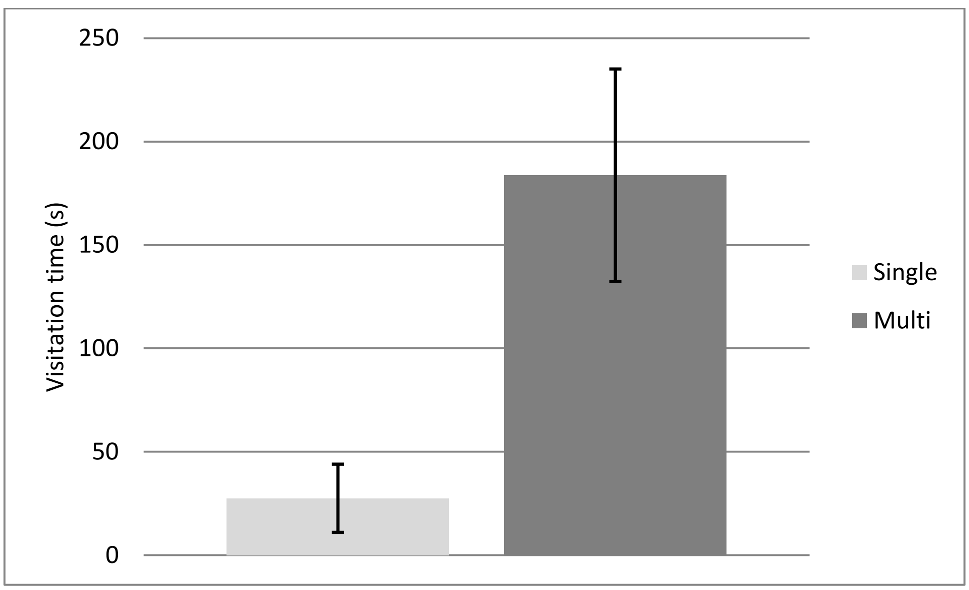

Figure 2: Average total visitation time for single- and multi-stemmed *Cirsium pitcheri* of Sturgeon Bay in Northern Michigan. Bars represent the mean ± standard error.

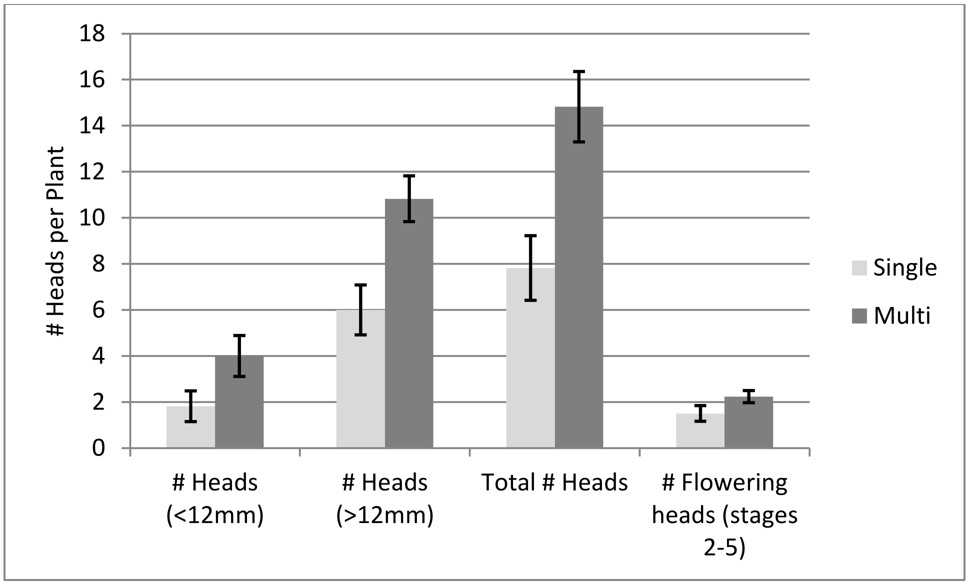

Figure 3: Average number of heads <12mm and >12mm, average total heads per plant, and average number of flowering heads per plant for single- and multi-stemmed *Cirsium pitcheri* of Sturgeon Bay in Northern Michigan. Bars represent the mean ± standard error.

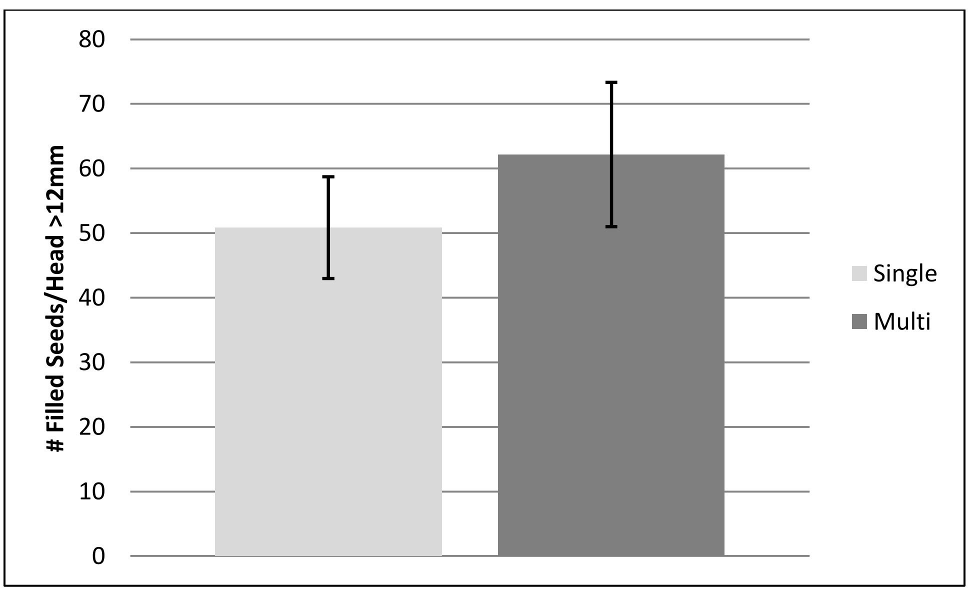

Figure 4: Average number of filled, viable seeds per large head >12mm for single- and multi-stemmed *Cirsium pitcheri* of Sturgeon Bay in Northern Michigan. Bars represent the mean ± standard error.

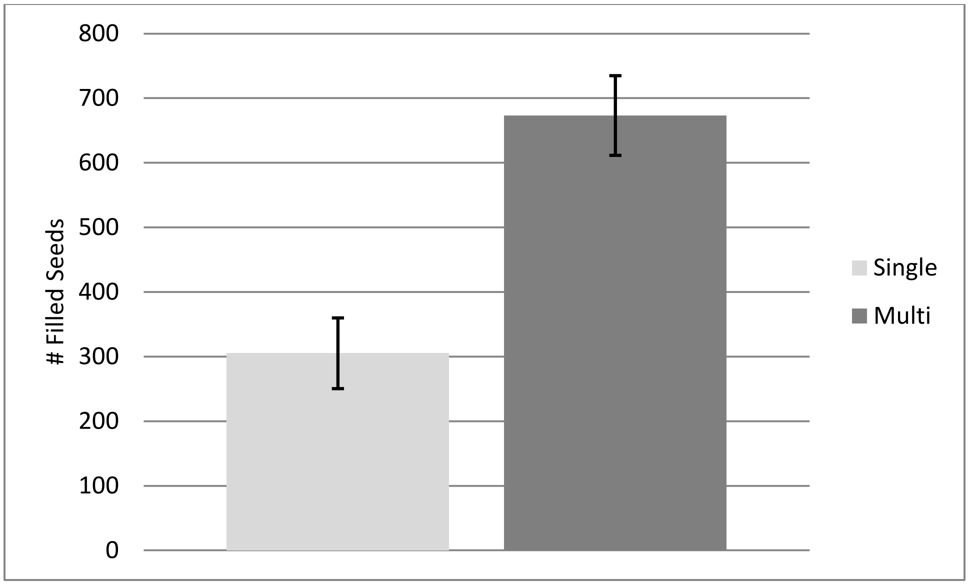

Figure 5: Average number of filled, viable seeds per plant for single- and multi-stemmed *Cirsium pitcheri* of Sturgeon Bay in Northern Michigan. Bars represent the mean ± standard error.

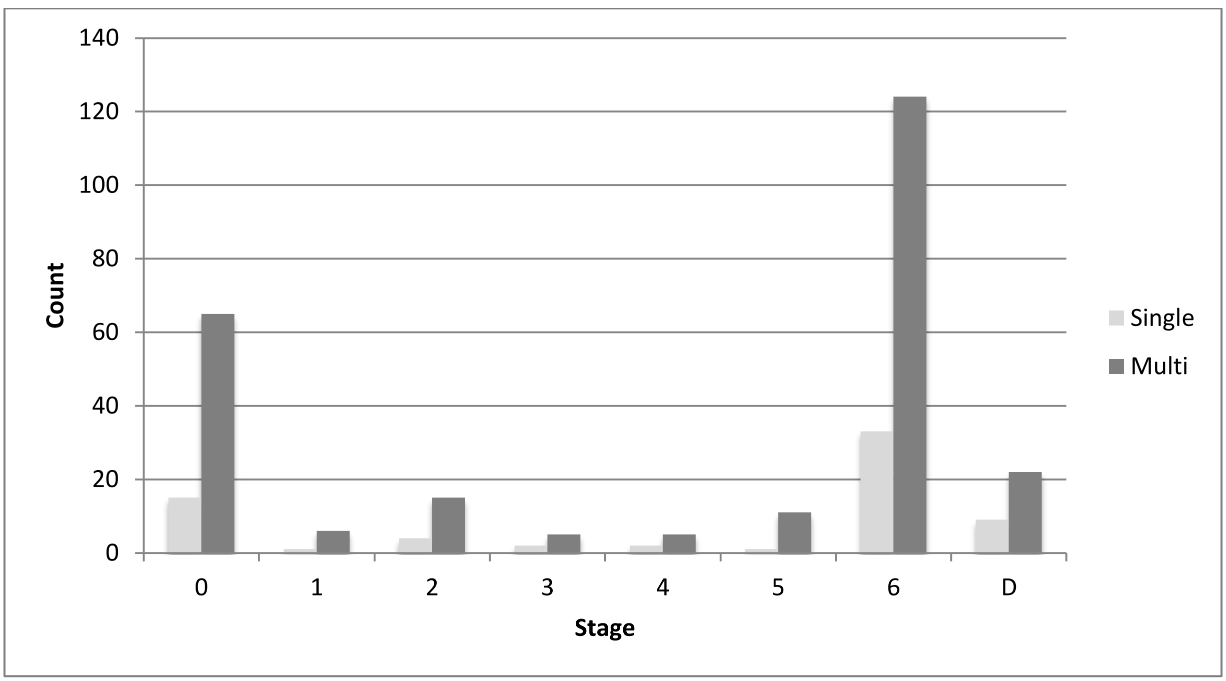

Figure 6: A comparison of flowering stage frequencies between single- and multi-stemmed *Cirsium pitcheri* of Sturgeon Bay in Northern Michigan. Zero represents a head that has not yet opened, one represents a head that has just begun to open, two to five represent flowering heads, six represents a head that is past flowering but has yet to disperse, and D represents a head that have already dispersed its seeds.