INDIRECT, PLANT MEDIATED INTERACTIONS OF MERISTEM MINERS ON FLOWER HEAD FEEDERS, A CASE FOR NON-INDEPENDENCE?

A Thesis by

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The following faculty members have examined the final copy of this thesis for form and content, and recommend that it be accepted in partial fulfillment of the requirement for the degree of Master of Science with a major in Biological Sciences.

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ABSTRACT

A long standing, dominant paradigm in the biological control of weeds is the independence of insect herbivore guilds' effects on their host plant. Recent work has shown stronger interactions among insect herbivore guilds than was previously expected. My research focuses on damage to apical meristems of tall thistles (*Cirsium altissimum*) by stem mining insects and the direct effects of this damage on plant architecture as well as the indirect, plant-mediated effects of this damage on flower head feeding insects and the arthropod predator community associated with tall thistle in south-central Kansas. Three questions are addressed; 1) What is the natural history of the predatory arthropod – tall thistle interaction in south-central Kansas? 2) Does apical meristem damage by insect herbivores influence host plant architecture, flower head damage and/or predatory arthropod densities? 3) Does plant architecture affect flower head damage and/or predatory arthropods? Two experiments were used to address those questions. The first experiment compared bolting tall thistles whose apical meristems were protected with insecticide with tall thistles whose apical meristems were not protected and were subject to herbivory. Damage to the apical meristem creates a plant that is shorter and has more flower heads than plants with the apical meristem intact and marginally increases primary branching. The second experiment modified the architecture of a bolting tall thistle with apical meristem damage back to a "pre-damaged" state by clipping lateral branches of the main stem. Neither experiment showed any significant difference in flower head damage severity or frequency, nor any differences in predatory arthropod densities among treatments. Through an overcompensation mechanism in the plant, meristem mining insects indirectly influence flower head feeding insects mediated by tall thistle. My results do not support the importation of multiple biological control agents for weed control.

INTRODUCTION

Insect herbivory is a strong selective force in plant evolution (Berenbaum 1983, Maurico and Rausher 1997, Maurico 2001, Craig et al 2007) and emerging results suggest that herbivory is important in limiting plant population size (Louda and Potvin 1995, Louda and Rodman 1996) and structuring community composition (Lubchenco 1978). Much of a plant's energy is invested in ways to deal with herbivory and many adaptations have been evolved, including compensatory growth, chemical defenses, and even volatile chemicals to signal predators, pollinators, and even other plants.

An issue that has plagued scientists for decades is why there is high variability in herbivore damage within or among host plant populations (Coley 1983, Marquis 1984, 1990). Many explanations for variation in herbivory have been suggested. For example, it has been shown that habitat-specific differences in resource availability drive differences in insect herbivory in populations of bittercress (*Cardamine cordifolia*) in the Rocky Mountains where plants in the sun were damaged more than shaded plants (Louda and Rodman 1996). In addition to variation in resource availability to plants, internal factors like genetic differences that could affect plant tissue chemical composition or concentration and morphological differences, as well as external factors such as predator/herbivore density or activity are the main categories of hypotheses for intraspecific variation in herbivore damage.

Variation in herbivory has been shown to have lifetime fitness consequences for host plants in some systems. For instance, inflorescence-feeding insects on Platte thistle (*Cirsium canescens*) decreased number of flower heads produced, seed output, seedling recruitment, and seedling recruitment to reproductive adults (Louda and Potvin 1995). Reduced lifetime fitness of a target plant can have an impact at the community level, inducing effects such as predator

mediated coexistence (Lubchenco 1978). Predator mediated coexistence is a phenomenon where competitively superior organisms coexist with competitively inferior organisms in the presence of a predator that reduces the competitively superior organism's fitness, thereby reducing their competitive influence. The impact that reduced lifetime fitness in host plants has on the community can be seen in many trophic levels. Reduced plant-plant competition, changes in herbivore densities and herbivore community composition, as well as changes in predator densities and community composition are only a few such impacts.

A long-standing, dominant paradigm in insect community ecology has been independence between different guilds of insects feeding on a plant (James et al 1992, see Denno et al 1995, Blossey et al 2001). A guild of insects is described as a collection of insect species that share similar feeding strategies or target prey, such as stem-boring moths and beetles, and flower head feeding flies and moths. The paradigm of independent effects of insect herbivore guilds is illustrated by the ideal free distribution hypothesis (Fretwell and Lucas 1970). This hypothesis states that resources in an ecosystem are divided up to decrease inter-specific competition.

More recently, it is becoming increasingly clear that there is non-independence of insect guilds that feed on different plant organs (Denno et al 2000, Denoth et al. 2002, Kaplan and Denno 2007). In other words, damage caused by one insect herbivore guild can have complex effects on the amount of damage imposed by a different guild. An example from this scenario would be a folivore removing photosynthetic tissue that changed the quality of pollen that affects a pollen feeder. This means that damage done by one guild of insects to a host plant can influence the severity of damage done by a different guild of insects. For example, root herbivory often causes physiological and/or morphological changes in a plant (Gange and Brown 1989). Masters *et al.* (2001) used *Cirsium palustre* for a study of indirect interactions between different

feeding guilds of insects. That study examined how a root feeding herbivore changed the chemistry of the plant making it more attractive to a seed-feeding tephritid fly. Increased tephritid fly densities, in turn, led to an increase in abundance of a parasitoid wasp, illustrating a non-independence of three insect guilds; two herbivorous and one insectivorous.

The independence vs. non-independence of insect herbivore guilds' damage and impact is important in the context of biological control for noxious weeds. Classical biological control depends upon the hypothesis that importation of the natural enemies of invasive species will lead to population control. Many argue that importation of multiple natural enemies will yield the greatest impact on the invader (Harris and Cranston 1979, McEvoy and Rudd 1993) without experimental support that multiple guilds of herbivores will have a greater impact on plant fitness than would a single highly effective herbivore.

There are diverse mechanisms by which non-independence of phytophagous insect guilds can arise. One is direct competition. For example, an insect that chews through the petiole of the leaf, thereby killing all leaf area distal to the damage will have direct influence on a leaf miner. Non-independence also could arise through plant-mediated indirect effects. This is best explained as morphological, phenological or chemical changes in a plant in response to damage by one guild that either increases or decreases the plant's attractiveness to a different guild. For example, Soler et al. (2007) describes an interaction where damage by the root feeding maggot *Delia radicum* changed leaf tissue quality in the host plant *Brassica nigra* that attracted a parasitoid wasp *Cotesia glomerata*. The wasp then parasitized the leaf-feeding caterpillar *Pieris brassicae*. The greatest effect was achieved when a neighboring plant, not the host plant, was root damaged. The damage to the neighbor reduced searching time of *C. glomerata* in finding its caterpillar host on *B. nigra*, regardless of host plant damage. Once the wasp chose an area

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without neighboring root damage, they parasitized the leaf feeder on the host plant more often. It is believed that this extra attractiveness of the host plant results from a volatile secondary compound produced in reaction to the root herbivory.

The architectural complexity of a plant is important in mediating insect herbivore-plant interactions in many instances (Boege 2005, Langellotto and Denno 2006). In a study by Riihimaki *et al.* (2006), complex trees, trees with a higher number of shoots, increased the survival of moth larvae most likely through the ability of the herbivore to hide from the predator. Earlier herbivore damage may affect subsequent damage by a different herbivore guild not only by changing plant chemistry, as demonstrated by Soler et al. (2007), but also by changing the morphology or architectural complexity of the host plant. For my work on tall thistle (*Cirsium altissimum*), architectural complexity will be defined in two ways; 1) by number of primary branches, or branches originating from the primary stem, 2) number of flower heads.

The strength of tri-trophic interactions involving insectivores, herbivorous insects and plants in determining plant reproductive success has been a topic of intense debate in the study of plant-animal interactions (Williams et al 2001, Griffin and Thaler 2006, Riihimaki et al 2006, Soler et al 2007, Miller 2008). Tri-trophic interactions refers to a situation where three trophic levels are involved, in this case a producer trophic level (host plant) influences a primary consumer (herbivorous insects) that in turn influences a second order consumer (predacious arthropods). Griffin and Thaler (2006) showed how a predators' presence not only affected the density of herbivores on plants, but also affected the behavior of herbivores. The authors quantified the amount of damage done to the plant and the plant's production of peroxidase, which is a damage-induced secondary defense compound that is costly for the plant to produce. The presence of predators reduced both damage and peroxidase production. Predator-caused

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reductions in herbivore densities have been shown to impact not only damage to host plants, but also to impact host plant performance. Floyd (1996) showed that two different predator guilds, birds and insect predators, decreased herbivore abundance and therefore increased plant growth on creosotebush (*Larrea tridentata*). Trickle down effects of predators on plant reproduction have been shown by Snyder and Wise (2001), where the presence of both a spider (Lycosidae) and a predacious beetle (Carabidae) increased cucumber fruit yield in an agriculture field.

The goals of my thesis research are three fold; 1) to describe the natural history of predatory arthropods on tall thistle in south-central Kansas 2) to understand the relationship between tall thistle plant architecture and the amount of flower head damage to the plant, and 3) to determine the influence of reproductive tall thistle architecture on predatory arthropod density associated with the plant. Questions that I addressed are; 1) What is the natural history of the predatory arthropod – tall thistle interaction in south-central Kansas? 2) Does apical meristem damage by insect herbivores influence host plant architecture, flower head damage and/or predatory arthropod densities? 3) Does plant architecture affect flower head damage and/or predatory arthropods? This data will provide insight into the debate concerning independence vs. non-independence of damage by insect herbivores.

METHODS

Study Species

Cirsium altissimum (tall thistle) is described as a biennial to short-lived perennial herb that is 1 to 2.5 m in height and grows from a fleshy taproot. Its range is most of the eastern United States, as far west as the eastern 1/3 of North and South Dakota, most of Nebraska, the eastern half of Kansas and Oklahoma, and the eastern 1/4 of Texas (Great Plains Flora Association 1986). Tall thistle lives as a rosette for one or more years, then produces a reproductive stalk (this life-stage is referred to as a "bolter," this is the life-stage that my study focused upon) and dies after only one year as a bolter. Inflorescences, called "flower heads", are terminal on the branch tips, and found singularly. The florets (disk flowers) are dark to lighter purple, rarely white. The seeds, or achenes, are tan to brown with a yellow apical ring. Each achene has a pappus, which is a grayish-white, feathery down attached to the achene that provides a method of dispersal. It carries the seed on air currents away from the parent. Common habitats for tall thistle include roadsides, lowland areas adjoining fields, and waste areas. Generally, tall thistle flowering begins in late July and ends in October, or whenever the first hard frost occurs. The specific timing of flowering in Kansas has not been quantified as of yet.

Common types of insect herbivore damage to tall thistle that are of interest to my study are apical meristem mining, and flower head damage. The apical meristem is a small section of tissue at the apex of the primary stem of the flowering stalk stem that divides and causes the flowering stalk to elongate. This tissue plays a critical role in the architecture of the plant. Apical meristem tissue secretes auxin that is hypothesized to suppress lateral branching (Mauseth 2009). The mining is most likely carried out by the artichoke plume moth larvae (*Platyptilia carduidactyla*).

Flower head damage can come in many varieties and have diverse impacts. Damage to the pappus can reduce the distance of seed dispersal, possibly creating a higher intra-specific competition among parents and their off-spring that germinate from seeds that fall near the parent. The seeds can be damaged which will reduce their viability, or kill them outright. The receptacle base, which is the attachment point in the flower head for developing seeds, can be tunneled halting the development of seeds adjacent to the tunneling by severing vascular connections to the seeds. Any damage to the stem very near the flower head can lead to wilting, which causes abortion of that head and loss of any reproductive opportunity associated with that head. Insects responsible for these types of flower head damage are flies of the families Tephritidae and Otitidae as well as larvae of the sunflower moth (*Homeosoma sp.*).

Study Sites

My study was conducted at three sites in and around Wichita, Kansas. The Wichita State University Ninnescah Reserve (formerly field station, F.S.) (37"32'N, 97"40'W) comprising roughly 330 acres, is approximately 56 km southwest of Wichita, Kansas. Pawnee Prairie Park (P.P.) (37"38N, 97"26'W) is the largest of my three study sites at 660 acres, and is located in SW Wichita. Chisholm Creek park (C.C.) (37"44'N, 97"16'W) is the smallest study site at 282 acres, and is located in NE Wichita. The greatest distance between sites (F.S. to C.C.) is less than 50 km which should control for any weather related differences between sites.

All three sites are restored, reseeded prairie dominated by big bluestem (*Andropogon gerardii*), indiangrass (*Sorghastrum nutans*), and switchgrass (*Panicum virgatum*). Average annual rainfall for Wichita is 77.16 cm, although 2008 set an all time record for annual rainfall, topping 137 cm of precipitation and a two year record precipitation (2007-2008) of 233 cm

(http://www.crh.noa-a.gov/ict/scripts/viewstory.php?STORY_NUMBER=2009011120).   Woody

encroachment is rampant, since wildfires are controlled to protect human life and property.   All

three sites have prescribed burns at regular intervals to emulate natural disturbances and help

control woody encroachment. Among my three study sites, Pawnee Prairie was most recently

burned in spring of 2007 (Jim Mason, pers. comm).

Field Methods

The Louda lab at University of Nebraska-Lincoln devised a protocol for describing thistle

architecture using a numbering system (Fig. 1A) and I have adopted this protocol for my studies.

Figure 1. Examples of terminology used to describe bolting tall thistle architecture. A)

numbering system used to identify tall thistle flower heads and branches. B) explanation of

single stemmed vs. multi-stemmed C) explanation of plant height vs. stem height.
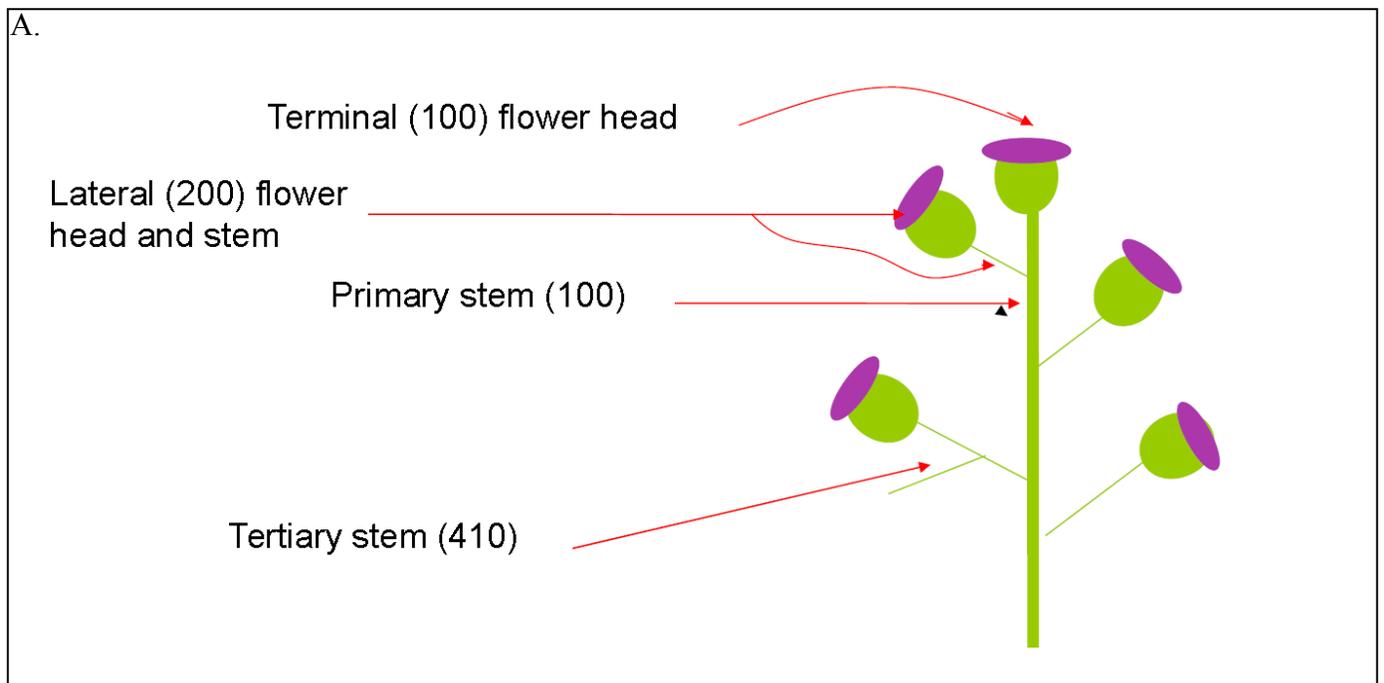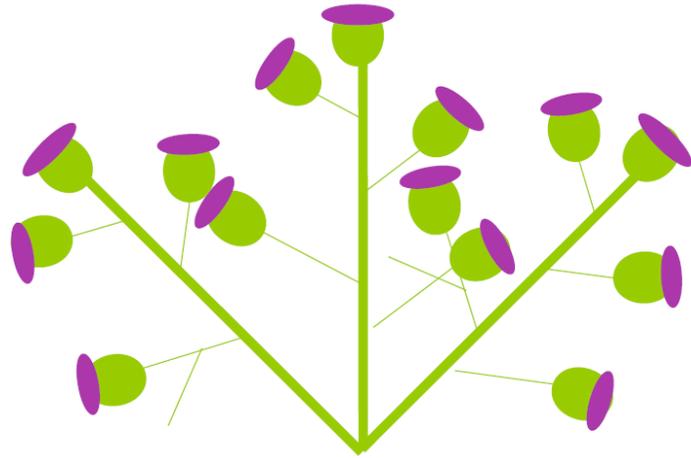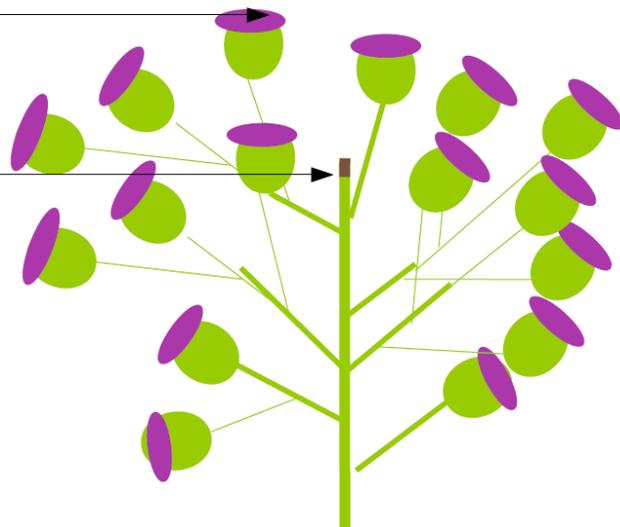
B.
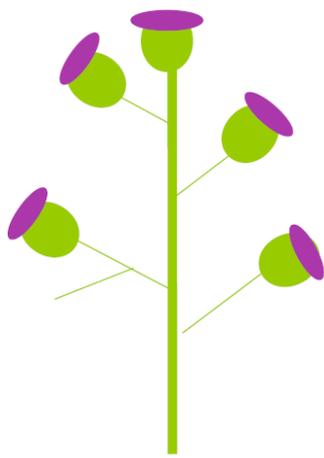
Single stemmed   vs.   multi-stemmed

C.

Plant height

Stem height

Starting at the apical meristem, the primary stem and associated flower head would be referred to as the "100" stem and flower head. All branches originating from the 100 stem (hereafter "primary branches") are numbered top to bottom as 200, 300, 400 and so on. Flower heads terminating on primary branches carry the same number as their primary branch. Any further branching off of a primary branch carries a lower designation, such as a branch off of the first primary branch would be called the 210 branch, with associated terminal flower head numbered 210 as well. Further branching has occurred, and continued the same pattern, 211, 211.1 and so forth.

On occasion, tall thistles may produce multiple stems that emerge from the same base (Fig. 1B). Multi-stemmed morphology is thought to result from damage to the rosette root crown. In this study, I only used plants with single stems. Apical meristem mining is very prevalent, occurring on nearly all tall thistles around Wichita, KS (FL Russell, personal observation).

Two experiments were performed in order to evaluate the interactions of the arthropod community on tall thistle, namely; stem feeding herbivores, flower head feeding herbivores, and insectivorous predators. In the experiments and the observational study, I used the same method of selecting bolting tall thistle plants with which to work. I made a transect that extended through a tall thistle population at each site and tagged the nearest single-stemmed tall thistle plant at 10 m intervals along the transect. Transect length at each site was 360 m for all experiments allowing a sample size of 12 plants per treatment per site (all experiments involved three treatments). Therefore, the total initial sample size for each experiment was 108 plants. Location of transects within each site was chosen based on occurrence of tall thistle, but otherwise was haphazard. For the two experiments, treatments were randomly assigned to

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bolting tall thistles in sets of three. In other words, tall thistle bolters at three adjacent sample points along the transect were grouped and treatments were assigned randomly among those three plants. This is to further avoid the risk of grouping any treatment together spatially. The insecticide used was Bifen IT (Control Solutions INC), a synthetic pyrethroid that is non-systemic and has no phytostimulatory effects, and stays active for only a few days after which reapplication is necessary. Dilution rate was approximately 1.7% or 17 ml of Bifin I/T/ insecticide per 1 L of water.

Experiment 1: Protecting Apical Meristem via Insecticide.

The purpose of this experiment was to examine effects of meristem mining on damage by the flower head feeding guild. I hypothesized that plant-mediated effects of the meristem-mining guild on the flower head-feeding guild might arise if apical meristem-miners alter plant architecture. Therefore, this experiment is a test of the independence of damage by herbivore guilds.

For this experiment, there were three treatments, 1) insecticide applied to the apical meristem, 2) water control and 3) no spray control. For plants that received insecticide, a very small amount, roughly a tablespoon, of insecticide mixture was applied to the apical meristem tissue during each round of insecticide application. Every effort is made to reduce the amount of insecticide coming in contact with any tissue other than the apical meristem, although trickling down the primary stem and a minimal amount of splashing were unavoidable. Water control consists of all the same actions as the insecticide sprayed plants, but, instead of applying insecticide, tap water was applied to account for any effects of the water in the insecticide.

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Control plants received no spray. Spraying occurred at a minimum of every two weeks, additional application was necessary if there was rain within 24 hours of application. Application of treatments began in late April in order to best control the stem mining insects. Treatment continued until florets began to be exerted on at least one flower head on the plant, but I then stopped application so as not to affect pollinators. Flower heads were collected for quantification of damage by flower head feeding insects in early October to ensure full maturation of viable flower heads. Some seed dispersal did occur before flower heads were collected, so seed counts could not be performed and lifetime seed production was not measured. All flower heads were collected simultaneously from a given site in order to allow uniform time for flower head feeding herbivores to feed.

Morphology variables that I quantified on my experimental bolting tall thistles included root crown diameter, stem height, plant height, presence of apical meristem damage, number of primary branches and number of flower heads. At ground level, tall thistle has an expanded root tissue called the root crown. It is believed this tissue is a storage organ. The diameter of the root crown has been shown to be a good indicator of plant resources, and strong indicator of potential plant size. Root crown diameter is measured by inserting calipers into the ground at the stem-ground interface. The height of the plant is measured from the ground to either the tip of the 100 stem, hereafter "stem height," or the highest point of the plant, hereafter "plant height" (Fig. 1C). Apical meristem damage was a categorical measurement, either "mined" or "no mining." Morphology measurements occurred three times for the spraying experiment, at the beginning of the experiment (8-10 April 2008, 28-30 April 2009), mid-season (3-5 June 2008, 23-25 June 2009), and just prior to flower head collection (1-3 October 2008, 19-21 October 2009). Flower heads were dissected in the lab to rank the magnitude of internal insect damage (Table 1).

Table 1. Categories used to rank tall thistle flower head development and damage. * denotes flower head stage categories collected and used in damage assessment.

Flower head stage categories 1 = small bud 2= large bud 3= early flowering (≥1 floret exerted) 4= late flowering (all florets exerted)* 5= mature (all florets have lost color)* 6= dispersing*	Receptacle base inflation 1=0-1mm 2=1-2.5mm 3=2.5-4mm 4=4+mm 5= unattached	Receptacle base color 1= golden 2= light brown 3= dark brown 4= black, granular 5= unattached	Receptacle base tunneling 1= single small hole 2= several small holes 3= more than 33% of the receptacle base tunneled.
Receptacle base percent damage 1= <10% 2= 10-30% 3= 30-60% 4=60-80% 5=>80%			

"Receptacle base inflation" is defined as any increase in the thickness of the receptacle base, which in an undamaged flower head should be no more than 1-2 mm thick. "Receptacle base color" refers to the color of the surface of the receptacle base. This color may indicate many different types of damage, including seed damage, tunneling and possibly infection. "Receptacle base tunneling" refers to a specific type of damage quite common in tall thistle flower heads where an insect larva chews a tunnel the diameter of the larva through the thickness of the receptacle base. Tunneling damage is impossible to see unless the flower head is cut in half. This particular damage is quite harmful to flower head seed production so categories represent a seemingly small percentage of the receptacle base tunneled. Finally, "receptacle base percent damage" is an overall assessment of the percentage of the receptacle base surface with obvious damage, taking into account receptacle base surface discoloration or damage, tunneling, and

inflation. This particular damage metric is most informative about damage to seeds. Seeds themselves often were no longer within the flower head at the time of collection.

Experiment 2: Modifying Damaged Plant back to "Pre-Damaged State"

To directly assess the effects of tall thistle architectural complexity on damage by the flower head feeding guild and the predator community on bolting tall thistles, I modified the architecture of plants that had suffered apical meristem damage plants back to a "pre-damaged" state by clipping off young primary branches.

For the branch-clipping experiment, only plants with apical meristem damage were used. Three treatments were assigned to these experimental plants; 1) unmodified control, 2) slight complexity reduction, and 3) high complexity reduction. Based on Louda and Russell's data from Nebraska on tall thistle, plants sprayed with insecticide over all tissues had significantly fewer primary branches, an average of three. Unsprayed control plants had an average of 16 primary branches. Based on those findings, for the high complexity reduction plants I clipped off all but the top three branches and for moderate complexity reduction plants I clipped off all but the top six branches. The intention of the moderate complexity reduction treatment was to provide an intermediate level of architectural complexity, simulating a plant that escaped apical meristem damage for much of the season, but incurred damage relatively late in the growing season. Branches were clipped off mid-season (14-16 July 2008, 13-15 July, 2009). Branches were removed using a pocket knife, cutting the branch as close to the stem as possible making sure to not cut the stem. Branches at this point in the season were very small, less than 5 cm long, most less than 1 cm. Re-growth of the cut branches did not occur during observation.

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Morphology measurements were the same as those described in the spraying experiment. Flower heads were collected simultaneously as with the spraying experiment and dissected and classified for insect herbivore damage using the same protocol as explained previously.

Predator Observation

During 2009, I repeatedly censused (17 August – 9 October) predators on tall thistle plants in both experiments. Observations occurred from 5-7 PM, and each site was visited twice per week for 16 observation periods per site. During each census I visually counted arthropod predators for two minutes per plant. I approached the plant, started the timer, walked a circle around the plant for approximately 45-60 seconds and then remaining time was spent close to the plant looking at the more hidden areas of the plant, such as under leaves and under flower heads. Identification of arthropods was made to family.

Statistical Analysis

Efficacy of Experimental Treatments in Experiments 1 and 2

All statistics were performed using SAS (version 9.1). In order to test the efficacy of insecticide application to bolting tall thistles' apical meristems in preventing meristem mining, a contingency table analysis was used for experiment 1 to test whether apical meristem mining affected a smaller proportion of insecticide-treated plants than control (water control and control combined) plants. Fisher's exact test was used for this contingency table analysis. A two-way ANCOVA was used for experiment 2 to test if clipping off of primary branches did, in fact,

reduce the number of primary branches compared to the unclipped control. Treatment was a fixed effect, site was a random effect and root crown diameter was the covariate.

For these analyses of experimental treatments' efficacies (proportion of plants with undamaged apical meristems for experiment 1 and number of primary branches for experiment 2), all plants that survived to be harvested were included in the analyses. An experimental plant might have been excluded from analysis if it was cut off approximately 3 cm above the ground (most likely white tailed deer herbivory), flooded and died, or killed by herbivory from below ground, most likely by burrowing mammals. For all other tests, only plants that conformed to the treatments imposed, such as insecticide applied plants with apical meristem intact, were used in the analysis.

In order to evaluate whether data conformed to the assumptions of ANOVA, I ran Levene's test for heteroscedasticity and evaluated normality using SAS Proc Univariate to compare observed residual distributions with those expected under a normal distribution. ANOVA assumptions were tested alike in all experiments. Dependent variables that were transformed to meet ANOVA assumptions were stem height, plant height and root crown diameter by natural log transformation and number of primary branches and number of flower heads by square root transformation.

For all morphology variables in both experiments, which included number of primary branches per plant, stem height, plant height and number of flower heads per plant, two-way ANCOVAs were used. Treatment was a fixed effect and site was a random effect. Because each experiment was repeated over two years (2008 and 2009), the effects of year on root crown diameter ($F_{1,2}=8.49$; p= 0.1054), stem ($F_{1,2}=1.04$; p=0.3139) and plant height ($F_{1,2}=1.16$; p=0.3933) were tested, and found to be insignificant. Therefore both years were pooled. Two

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levels of control, untreated control and water applied control, were tested for significant differences in root crown ($F_{1,2}$=0.51; p=0.6069), stem height ($F_{1,2}$=5.30; p=0.0940), and plant height ($F_{1,2}$=1.52; p=0.2273), and none were found, therefore both control treatments were pooled.

For damage variables, two-way ANOVAs were used with treatment as a fixed effect and site as a random effect. I did ANOVAs to evaluate effects of treatment and site on two damage-related dependent variables: 1) average percent damage to flower head receptacle bases per tall thistle plant and 2) average degree of tunneling done to flower head receptacle bases per tall thistle plant. These dependent variables for damage are the most generalized, evaluating any differences in damage in the two most prevalent and biologically relevant damage metrics. I also did ANOVAs to examine effects of treatment and site on the proportion of heads per plant both escaping tunneling and having the greatest damage score possible. These tests look at the both ends of the spectrum. Proportion of heads per plant escaping tunneling shows plants that had the greatest proportion of heads escaping the most destructive damage. Proportion of heads per plant having the greatest damage score shows plants that had the greatest proportion of heads that are damaged to the point of no longer being viable. For experiment 2, when a significant effect of treatment was found a Tukey test was used to identify the levels of the branch clipping treatment that differed significantly from one another.

For predator census analysis, contingency table analyses were used. I did Fisher's exact test to determine if there was any difference in the occurrence of a predator on a tall thistle plant between treatments. Dependent variables were presence/absence of arthropod predators per plant. Due to low numbers of predators, Fisher's exact test was used because Chi^2 was insensitive to such low numbers (Sokal and Rolf 1995). Each plant was assigned to one of the two

categories, either having at least one predator at one point during a census, or not having a predator at any point during a census.

RESULTS

Experiment 1: Effects of Apical Meristem Mining on Plant Morphology, Flower Head Damage and the Arthropod predator Community.
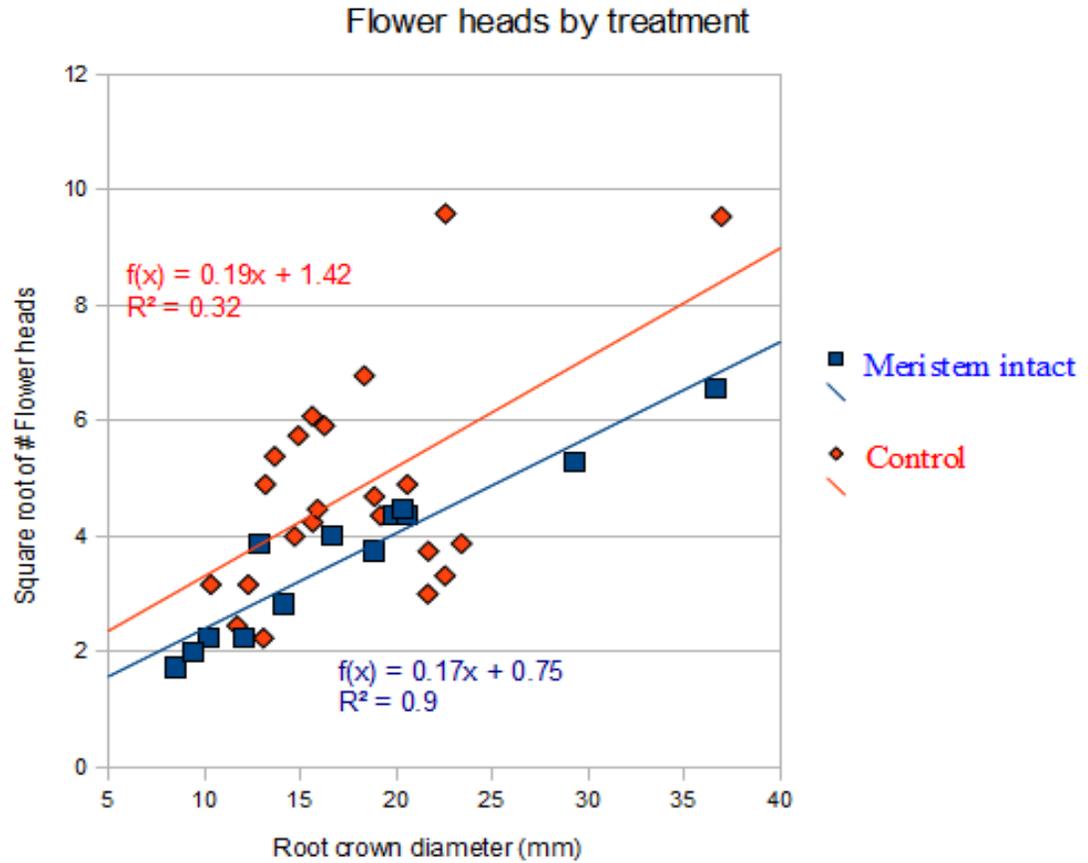
Efficacy of Insecticide Application

Plants whose apical meristems were sprayed with insecticide were significantly less likely to have their apical meristem damaged by insects ($p=<0.0001$) than were water control and control plants combined. 13 of 20 insecticide plants had their apical meristem intact and only 3 of 39 control plants had their apical meristem intact at the time of flower head harvest.

Effects on Tall Thistle Size and Architecture

There was no significant difference in final root crown diameter between treatments of plants ($F_{1,2} =3.83$; $p=0.178$). This ensures that results found were due to an effect of treatment, and not a pre-existing size difference among treatments.
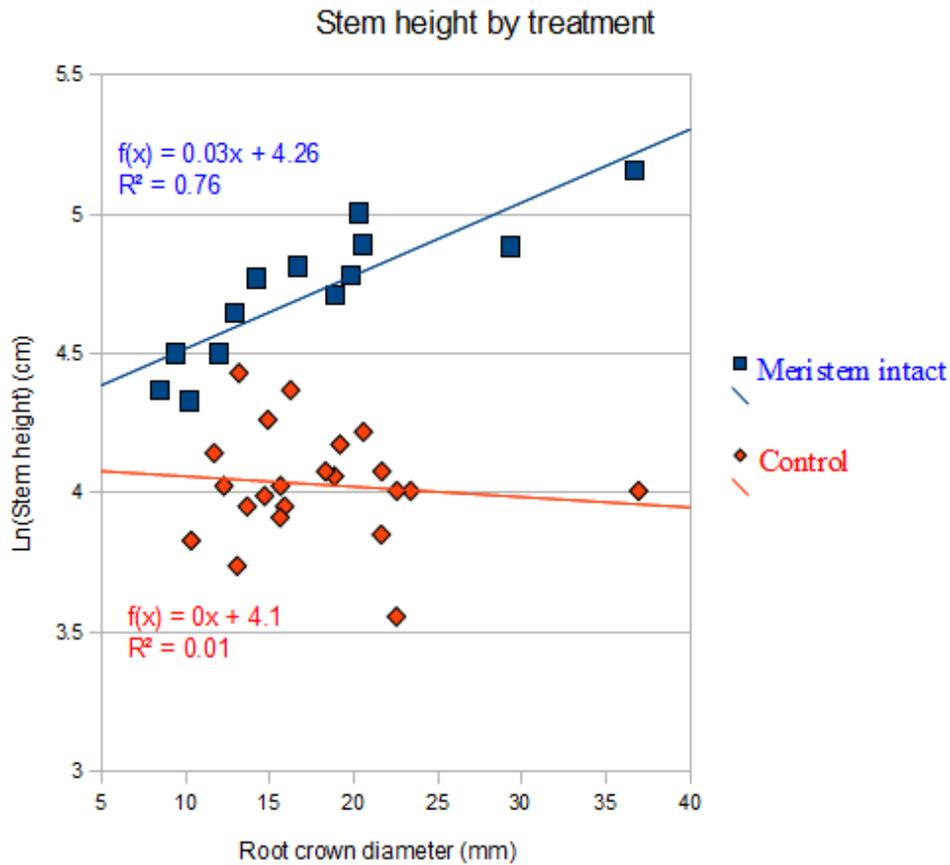
The number of flower heads per plant was significantly and positively related to root crown diameter (Figure 2; $F_{1,2}=4.10$ $p=0.0483$). Although insignificant as only a treatment effect ($F_{1,2}=2.24$ $p=0.1411$), with the co-variate accounting for plant size, plants with their apical meristems protected had significantly fewer flower heads than control plants.

Figure 2. Number of flower heads per bolting tall thistle plant in relation to whether the apical meristem was protected from meristem mining and as a function of root crown diameter.

Flower heads by treatment

f(x) = 0.19x + 1.42
R² = 0.32

f(x) = 0.17x + 0.75
R² = 0.9

■ Meristem intact

◆ Control

Square root of # Flower heads
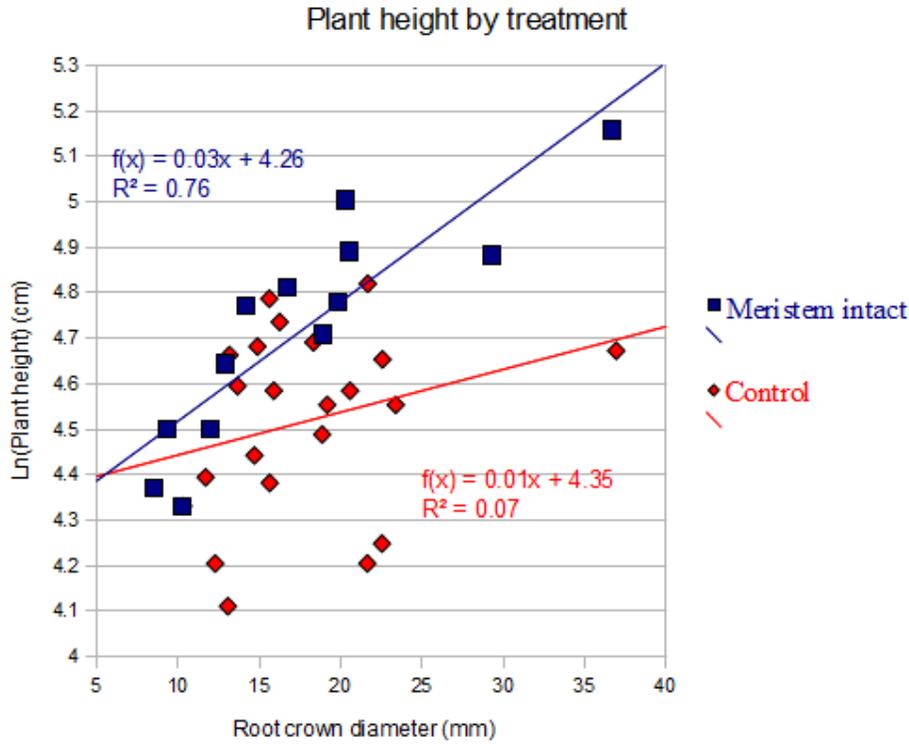
Root crown diameter (mm)

Once adjusted for size using root crown diameter, apical meristem protected plants had a significantly longer stem height than control (Fig. 3; $F_{1,2}$ =99.07; p=<0.00001). The stem height was also significantly and positively related to root crown diameter ($F_{1,2}$=5.11; p=0.0309)

Figure 3. Stem height of bolting tall thistle in relation to whether the apical meristem was protected and as a function of root crown diameter.

Stem height by treatment

f(x) = 0.03x + 4.26
R² = 0.76

Meristem intact

Control

f(x) = 0x + 4.1
R² = 0.01

Ln(Stem height) (cm)

Root crown diameter (mm)

Once adjusted for size using root crown diameter, apical meristem protected plants were significantly taller than control (Fig. 4; $F_{1,2}$=9.70; p=0.0040). Plant height was significantly and positively related to root crown diameter ($F_{1,2}$=15.63; p=0.0004).

Figure 4. Plant height of bolting tall thistle in relation to whether the apical meristem was protected and as a function of root crown diameter.

Plant height by treatment

Apical meristem protected plants had marginally significantly fewer branches than control plants ($F_{1,2}$= 3.20, p=0.0835). Number of branches was significantly and positively related to root crown diameter ($F_{1,2}$= 16.53; p=0.0003).

Effects on Magnitude of Tall Thistle Flower Head Herbivory

For the mean percent damage to flower head receptacle bases per tall thistle plant, there was no significant difference between plants with the apical meristem in-tact and control plants (Fig. 5; $F_{1,2}$=0.02, p=0.9029). Similarly, apical meristem protection did not affect the mean tunneling score of flower head receptacle bases per plant ($F_{1,2}$=0.00, p=0.9830).

Figure 5. Average percent damage to flower heads per plant in relation to whether the apical meristem was protected. Error bars indicate one standard error.

Average Percent Damage by treatment
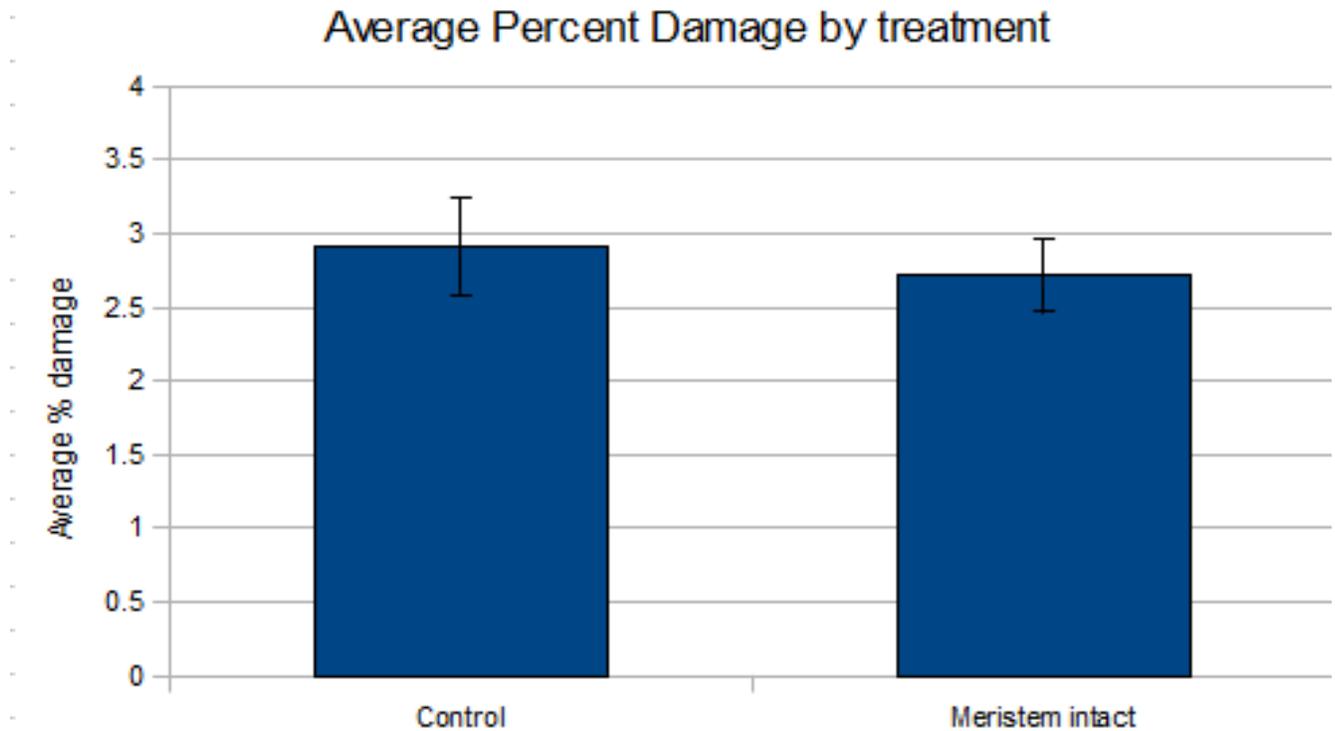
Protecting the apical meristem with insecticide did not affect either the proportion of flower heads per plant that escaped any tunneling insect herbivore damage (Fig. 6; $F_{1,2}=0.11$, p=0.7375) or the proportion of flower heads per plant that suffered the highest damage grade (Fig. 7; $F_{1,2}=0.12$, p=0.7334). Highest damage grade is a combination of all damage categories where the flower head had a receptacle base detached from the rest of the flower head, a black/granular color, percent damage >80%, and >33% tunneling.

Figure 6. Proportion of flower heads per bolting tall thistle that escape tunneling in relation to whether the apical meristem was damaged. Error bars indicate one standard error.
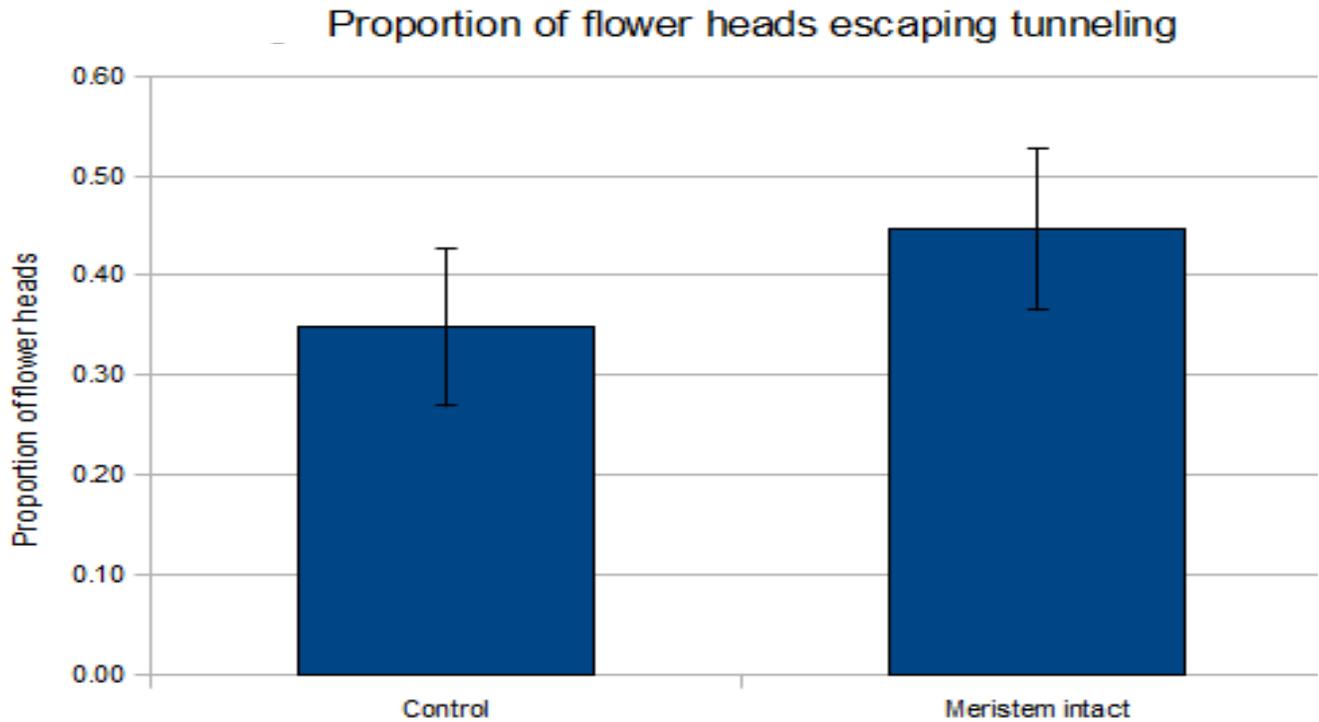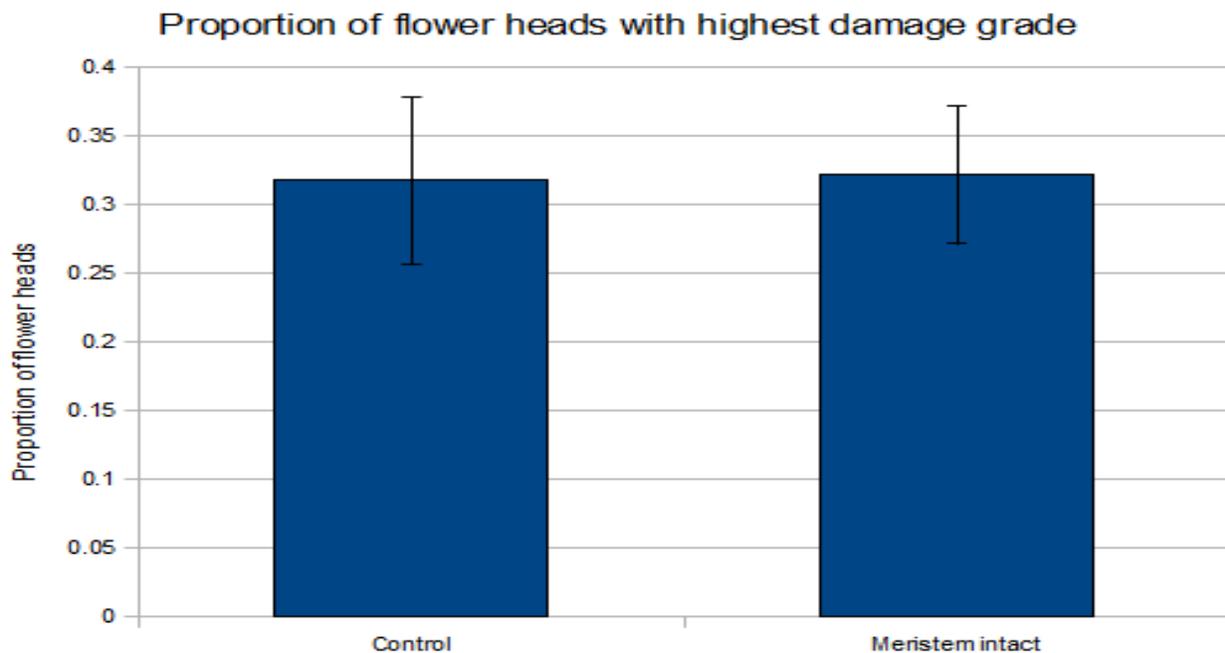

Figure 7. Proportion of flower heads per bolting tall thistle that receive the highest damage grade in relation to whether the apical meristem was damaged. Error bars indicate one standard error.

Proportion of flower heads with highest damage grade

Effects of Apical Meristem Mining on Arthropod Predator Community Abundance

Arthropods found included 10 orb weaver spiders (Araneidae), 6 jumping spiders (Salticidae), one crab spider (Thomisidae) and one cob web weaver spider (Theridiidae). Whether or not an arthropod predator occurred on a bolting tall thistle did not depend on protection of the apical meristem (p=0.7098). I found a very low density of predators on tall thistle. For control plants, I found approximately 1 predator for every 17 plants, and for sprayed plants I found approximately 1 predator for every 14 plants. Three of 39 plants sprayed with insecticide had a predator at some point during my observations whereas 4 of 69 control plants had a predator.

Experiment 2: Effects of Experimental Reduction in Tall Thistle Architectural Complexity on Flower Head Damage and the Arthropod Predator Community

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Efficacy of the Branch Clipping Treatment in Reducing Tall Thistle Architectural Complexity

There was a significant difference in branch density among treatment levels ($F_{2,41}$=12.40, p=<0.0001). Branch density (number of branches per unit root crown diameter) was significantly and positively related to root crown diameter ($F_{2,41}$= 10.43; p=0.0024). Control plants had significantly more branches per unit stem height than did high or low reduction (Tukey test: p=<0.0001 and 0.0179 respectively) Low reduction plants were not significantly different from high reduction plants (Tukey test: p=0.143). There is a marginally significant difference in number of flower heads among treatment levels ($F_{2,41}$=3.05, p=0.0583). There was no significant difference between control plants and plants that received moderate complexity reduction in number of flower heads (Tukey test: p=0.8734), but control plants had marginally significantly more flower heads than high complexity reduction plants (Tukey test: p=0.0587). Low reduction plants were not significantly different from high reduction plants (p=0.1969).

There was no significant difference in plant height among the treatment levels ($F_{2,2}$=0.03, p=0.9720). Plant height was significantly and positively related to root crown diameter ($F_{2,2}$=84.05; p=<0.0001).

Effects of Plant Architectural Complexity on Flower Head Herbivore Damage

No significant differences were found in the mean percent damage done to flower heads per plant between the different levels of the architectural complexity manipulation (Figure 8; $F_{2,2}$=1.06, p=0.4861).

Figure 8. Mean percent damage done to flower heads per plant between the different levels of the architectural complexity manipulation. Error bars indicate one standard error.

Mean Percent Flower head Damage by treatment
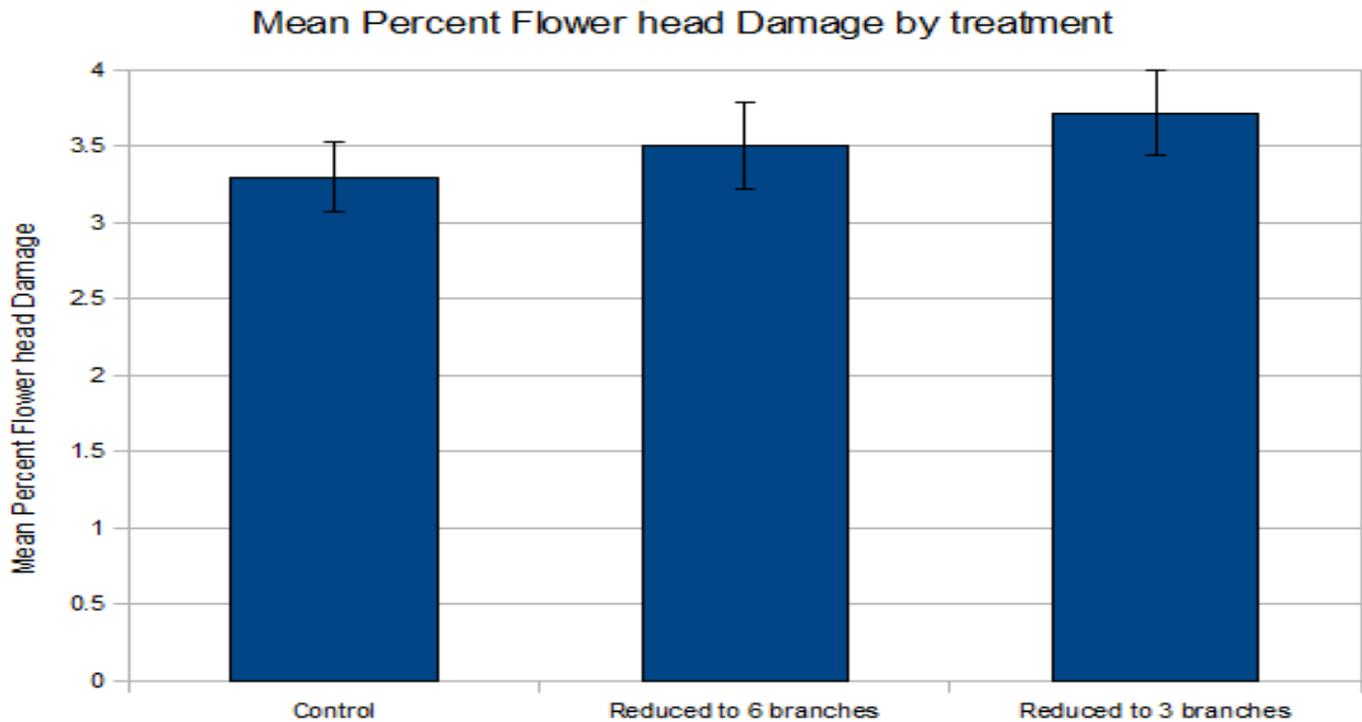
The mean amount of tunneling damage to flower heads on a plant did not vary with architectural complexity treatment (Figure 9; $F_{2,2}=1.6$, p=0.3851).

Figure 9. Mean tunneling damage to flower heads among complexity reduction treatments. Error bars indicate one standard error.

Mean degree of tunneling per plant by treatment
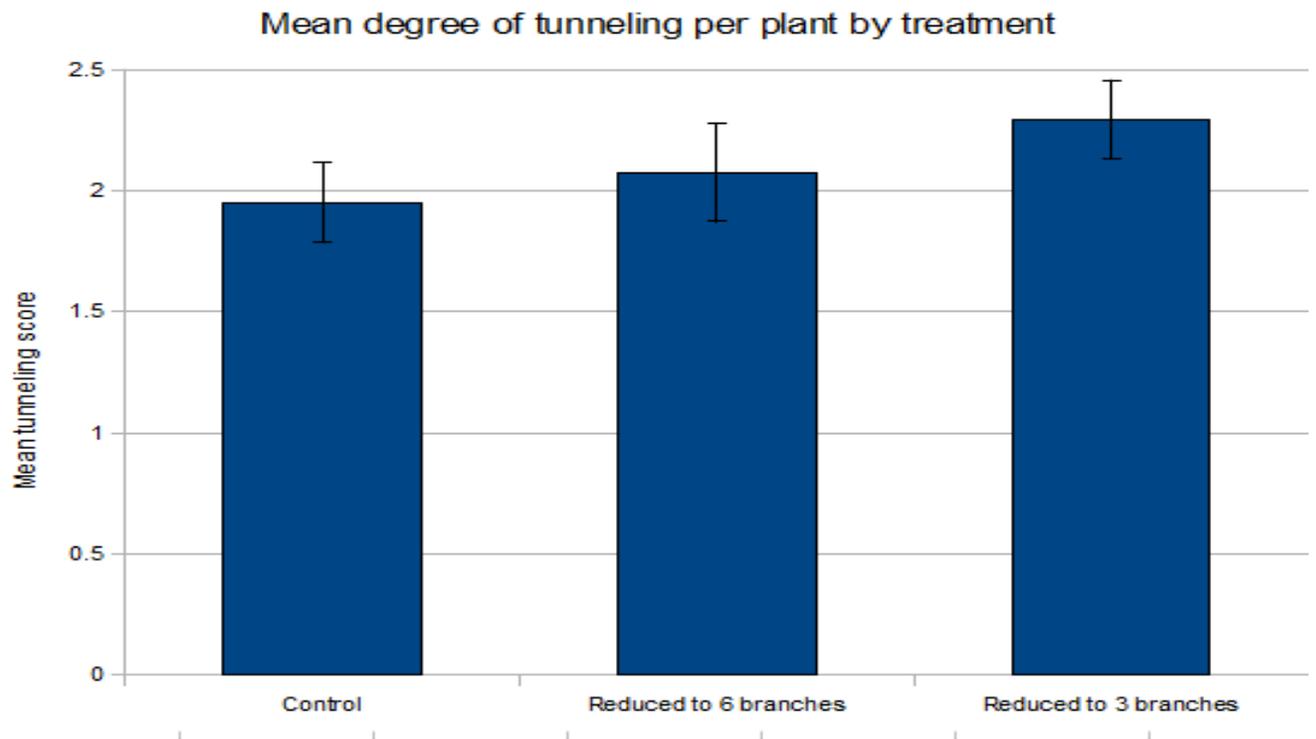
Proportion of flower heads that escaped tunneling was not significantly different among treatments (Figure 10; $F_{2,2}$=0.74; p=0.4850).

Figure 10. Proportion of flower heads that escaped tunneling among levels of complexity reduction treatment. Error bars indicate one standard error.

Proportion of Flower heads escaping tunneling per plant by treatment
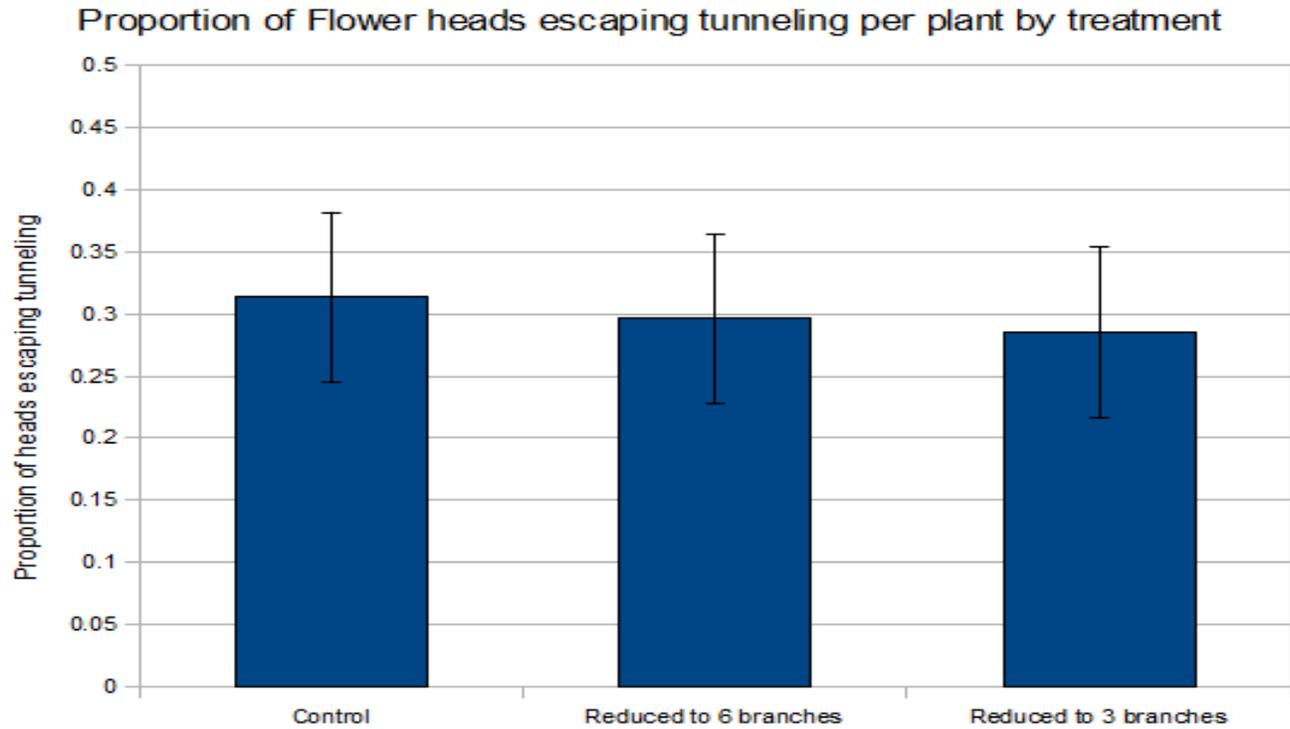
Proportion of flower heads receiving the highest damage grade also did not differ significantly among treatments (Figure 11; $F_{2,2}=0.14$; $p=0.8678$).

Figure 11. Proportion of flower heads receiving the highest damage grade among levels of complexity reduction treatment. Error bars indicate one standard error.
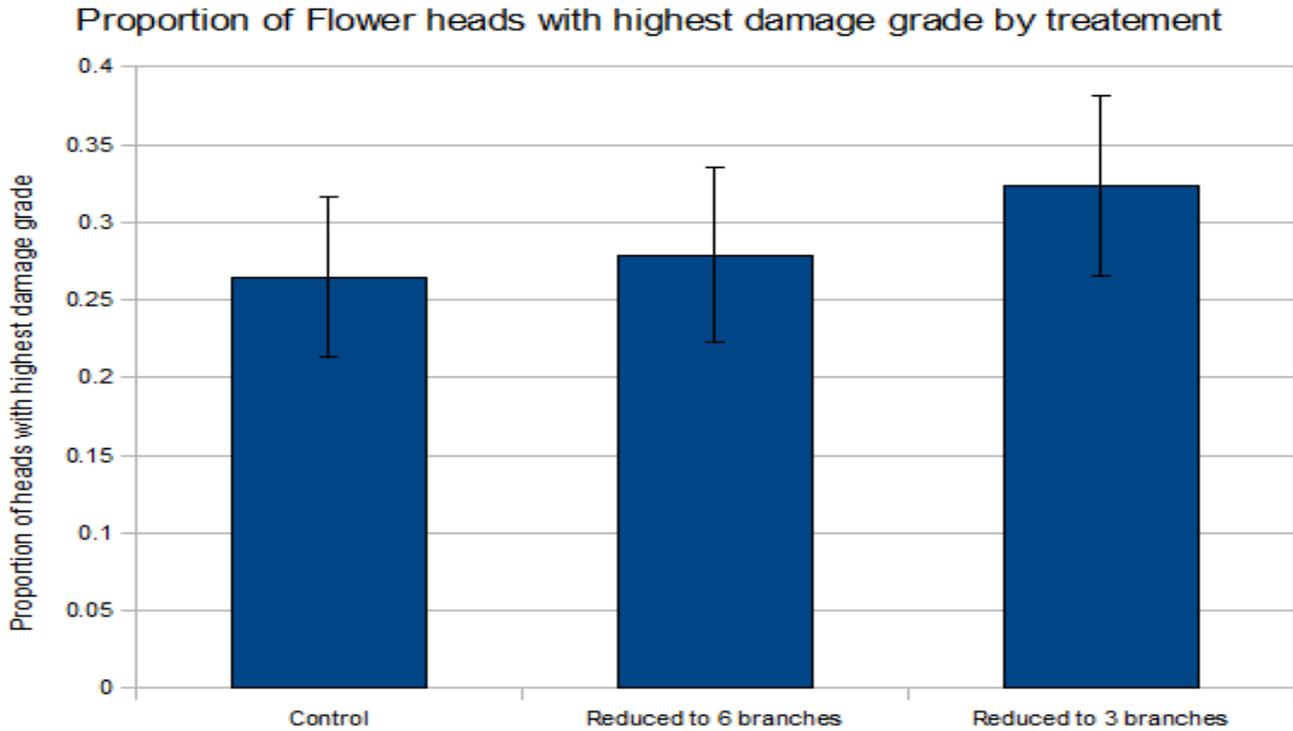
Effects of architectural complexity on predator community abundance

Predatory arthropods found include 8 orb weaver spiders (Araneidae), 4 jumping spiders (Salticidae), 2 crab spiders (Thomisidae), one cob web weaver spider (Theridiidae), and a damselfly (Coenagrionidae). The damselfly was most likely a transient, and not using the tall thistle as a hunting ground as they are mostly aerial hunters. The density of predators was very low. I found approximately 1 predator per 3 plants on control plants, 1 predator per 5 high reduction and low reduction plants. There was no significant difference in number of plants with a predator (Fisher's exact test: p=0.7098) with 2/19 control plants having a predator, 2/19 moderate complexity reduction plants, and 4/20 high complexity reduction plants having a predator.

DISCUSSION

In summation, apical meristem mining affected many aspects of plant architecture. Damage to the apical meristem creates a plant that is shorter and has more flower heads than plants with the apical meristem intact. There was also a marginally significant result for the number of branches per unit root crown diameter, suggesting a weaker effect. These pronounced changes in bolting tall thistle architecture, however, did not impact damage done by the flower-head feeding insect guild. The apical meristem mining guild of insects, through an overcompensation mechanism in the plant, appears to increase the resource base for flower head feeding insects by increasing the number of flower heads per plant. This is accomplished by making the plant shorter and having a higher density of flower heads per plant, thereby giving flower head feeding insects a larger food pool. Neither the intensity of damage per head nor the proportion of heads damaged by flower head feeding insects per plant, however, seems affected by the increase in number of flower heads per plant. This suggests that stem mining insects actually increase the number of flower heads that escape damage and thereby increase reproductive effort.

Effects of Meristem-Mining on Bolting Tall Thistle Architecture

It is feasible to perform an observational study of the impacts of apical meristem mining on plant architecture and the plant's arthropod community by comparing plants that naturally escape apical meristem mining with plants that naturally receive mining. Such an observational study would not definitively identify the cause of any differences in morphology, damage or the

herbivore and predator community between meristem-mined plants and plants that escaped meristem mining. Possible factors that might be confounded with meristem mining in an observational study include; genetic variance that caused different secondary chemistry, leaf toughness, or even timing of bolting and flowering, associational resistance from neighboring plants, even insect choice. The only way to properly address causation is through experimentation.

Many studies have compared plants that naturally experienced meristem mining with plants that naturally escaped mining, (Inouye 1982, Clark and Clark 1985, Maschinski and Whitham 1989, Banta et al 2010) while other studies have manipulated meristem mining through artificial meristem damage (Argall and Steward 1984, Sheldon 1986, Benner 1988, Michaud 1991, Wise and Abrahamson 2008). Morphological effects of apical meristem damage are reported to include increases in fruit set and seed production (Paige and Whitham 1987), increasing shoot production (Heichel and Turner 1983), and many others. My findings support previous work, in that apical meristem damage increased the number of reproductive structures produced, here flower head number, per plant (Paige and Whitham 1987), although most other metrics that I compared between damaged and protected tall thistles were insignificant or decreasing with damage. Maschinski and Whitham (1989) suggested that an entire range of plant compensatory responses to herbivore damage are possible, given certain conditions apply, such as the identity of the plant species in question, the intensity and timing of damage, and levels of competition and nutrient availability. My study did not consider many of the environmental conditions that might explain variation in any given range of responses to apical meristem damage. My study does, however, take a novel approach to addressing a previously overlooked issue; the experimental prevention of apical meristem damage.

An unavoidable criticism of simulated herbivore damage imposed by an investigator is that damage done by an insect is difficult if not impossible to replicate. The chewing mouthparts of insects are incredibly difficult to mimic due to their size and composition. Also, the addition of saliva during chewing is very likely and can introduce the possibility of disease transmission (Cipollini 2002), which is nearly impossible to replicate. Other forms of damage, such as mining can be even more complex, since the insect often will grow within its host plant, continuing to feed and also defecate within the wound (personal obs.). Clipping off apical meristem tissue hardly replicates this.

While my second experiment involved clipping off tissue, the clipping was done to modify architecture rather than to simulate herbivory. Most other studies have clipped apical meristem tissue as "simulated apical meristem damage." I have let apical meristem damage occur, and modified the plant, at least in architecture, back to a state similar to a plant protected from damage (Russell unpublished data).

Interestingly, damage to the apical meristem produced a rather poor correlation between the number of flower heads and the root crown diameter (R^2=0.32), while protecting the meristem produced very high correlation (R^2=0.9). Similarly, the correlation between stem height and root crown diameter was very low for meristem damaged plants (R^2=0.01), while being very high (R^2=0.76) for meristem protected plants. This suggests that damage to the apical meristem may release the plant from normal allometric constraints for multiple morphological dependent variables. I believe this may be due to the overcompensation mechanism built into the plant, suggesting a long, tightly associated history between tall thistle and meristem boring insects. Overcompensation can mean many things. For my study, I am defining overcompensation as "any response to tissues lost due to herbivory greater than control." This is similar to a definition

used by Maschinski and Whitham (1989). Whether or not the overcompensation in flower head production results in an increase in fitness cannot be determined from my study. To properly address fitness, one must quantify lifetime seed production, seedling establishment, and seedling lifetime seed production. I have quantified lifetime flower head production, since tall thistle is monocarpic.

Given the large number of studies that have shown increases in fruit, seed, and flower production in response to apical meristem damage (Heichel and Turner 1983, Paige and Whitham 1987, Maschinski and Whitham 1989) it would be improper to assume the increase in flower head number found in this study is only explained by an evolutionary response to apical meristem mining. Instead, it may be a physiological response to release of apical dominance.

Effects of Bolting Tall Tistle Architecture on Flower Head Damage and the Arthropod Predator Community.

Experimental reductions in the architectural complexity of bolting tall thistles (e.g. branch clipping) had no effect on the severity of damage by the flower head feeding guild of insects associated with the plant. Perhaps plant architectural complexity did not affect damage by the guild of flower head feeding insects because differences in architectural complexity did not affect the density of arthropod predators on tall thistles (Langellotto and Denno 2004). Other studies (Riihimaki *et al.* 2006) have shown that increased architectural complexity has led to increases in predators. I believe my findings on the contrary are due to inherent differences in the two systems studies, Riihimaki *et al.* (2006) examined the arthropod predator community on a tree (*Betula spp*), whereas I worked with an herb. Trees have a much wider range of possible

changes in architecture available than do herbs, and so therefore are more sensitive to the arthropod predator's density response. It is possible that my highest architectural complexity is still too low for an increase in arthropod predators. A lack of response in the arthropod predator community in my study is possibly due to an insufficient difference in plant architecture.

Although significantly different morphology traits were found (flower head number, stem and plant height) the amplitude of those differences may have been too small to see a substantial change in the arthropod predator community on bolting tall thistles. Other possible mechanisms that could affect the magnitude of insect herbivore damage include the "resource concentration hypothesis "(RCH) (Root 1973). This hypothesis states that patches of high resource availability should attract and hold higher densities of organisms exploiting that resource. Most commonly, the RCH is applied to patches of host plants for specialist insect herbivores. My study showed increases in density of flower heads, a concentrated resource, with no change in herbivore damage, many of whom are specialists. Although no data on herbivore species was collected, many of the flower head feeding insects are specialists (Takahashi 2006). One possible explanation as to why no increase in flower head damage was seen is that a change in flower head density on an individual plant is too small of a "patch" size to influence insect behavior. Possibly, only patches composed of many plants all with increased flower head density would be large enough to increase flower head damage.

Although not addressed in this study, bird predation may also impact flower head feeding insects as well as arthropod predators on tall thistle. Possible future work could include the effect of bird predation instead of just arthropod predation on tall thistle herbivores. A plant lifetime study, looking at actual lifetime fitness of the plant rather than just flower head number could give valuable insights. Also, many plants were lost to apparent white tailed deer (*Odocoileus*

virginianus) herbivory. It is possible that site selection could reduce that, possibly a site that allows deer hunting, or at least a site far enough outside of the city to reduce deer populations sufficiently.

Effects of Apical Meristem Mining on Damage to Tall Thistle Flower Heads – Assessing Insect Herbivore Guild Independence

Apical meristem mining by insect herbivores did not affect the magnitude of damage by flower head feeding insects. Flower head feeding insects damaged the same proportion of flower heads and did the same amount of damage to individual flower heads on average among all treatment levels. Previous work has supported non-independence of herbivore guilds and shown that defoliation by herbivores can significantly increase densities of subsequent sap feeding herbivores (Wallin and Raffa 2001). In a New Jersey salt marsh, an early-season species of phloem feeding planthopper greatly reduced the subsequent nitrogen concentration for a later feeding planthopper species, significantly affecting the second planthoppers survival and fecundity (Denno et al 2000). Other work has supported independence of herbivore guilds. Miller (2008) showed that on a cactus (*Opuntia imbricata*), a cactus bug (*Narnia pallidicornis*), was unaffected by a cactus beetle (*Moneilma appressum*). My work shows that regardless of previous stem mining, flower head feeding insects damage the same proportion of heads, which would seem to support independence. The indirect effect of the tall thistle on the later feeding flower head feeders shows an increase in flower head production, supporting non-independence. I believe that the more relevant conclusion to my results is non-independence between insect

herbivore guilds, plant mediated indirect effects of insect herbivores because of the possible increase in reproductive effort.

Comparing my result to the results of many other studies that address indirect interactions among insect herbivore guilds is difficult, because most other studies show only insect density indirectly mediated by plant responses. By contrast, instead of quantifying insect herbivore abundance, my study looks rather at damage intensity. I believe this difference in the dependent variables quantified may be one mechanism as to why my results support the older paradigm of independence of herbivore guilds, while many recent studies support the new paradigm of non-independence. My work looked at both interguild competition between herbivore guilds and the potential for top-down effects of predators, but did not include bottom-up competition for shared resources like Miller (2008). His study looked at the impact of a beetle which feeds year round on a bug that has multiple generations in a growing year. Since my study looked at temporally separate herbivores, direct correlations cannot be made, although results are similar. Plant tissue quality was not addressed in my study, so no comparisons can be made with studies that have looked at chemistry on subsequent herbivory (example, Wallin and Raffa 2001, Denno 2000). Nevertheless, the lack of difference in damage intensity to tall thistle flower heads in response to apical meristem mining would imply no change in plant chemistry, assuming density of flower head feeders per flower head remained the same.

The applied significance of this work is for designing biological control strategies to limit weedy plant performance. A common tenet of classical biological control has been that the importation of multiple herbivores, especially those that feed on different plant organs, will yield the greatest control of invasive exotic weeds (Myers 1984, James et al 1992, McEvoy and Coombs 1999, Blossey et al 2001). The importation of biological control agents is not without

dangers. The importation of *Rhinocyllus conicus* for control of musk thistle has led to the decline of a once common native thistle (*Cirsium canescens*) (Louda et al 1997). Many other biological control vectors have proven to be non-host specific (Pemberton 2000). Due to these risks associated with importing additional exotic insects for weed biocontrol, improved understanding as to whether attack on multiple plant organs by multiple biological control agents actually achieves greater suppression of the target weed, is crucial to the safe use of biological control. My work suggests that stem mining insects increase flower head production and, thereby, may increase seed production. Most importantly for tall thistle, flower head feeding insects show no increase in damage that would counter a potential increase in number of seeds.

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