EFFECTS OF INSECT HERBIVORY ON PLANT ARCHITECTURE, FLOWERING PHENOLOGY, FLOWER VISITORS' ACTIVITY AND REPRODUCTION SUCCESS IN CIRSIUM ALTISSIMUM L.

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Subodh Adhikari

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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.

_____
F. Leland Russell, Committee Chair

_____
Mary Liz Jameson, Committee Member

_____
Gregory Houseman, Committee Member

_____
William Parcell, Committee Member

DEDICATION

To my parents, my wife, my family and Wichita friends

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ABSTRACT

Insect exclusion experiments have demonstrated that insect herbivores can reduce host plant fitness through both direct and indirect mechanisms. I did an experiment on *Cirsium altissimum* (tall thistle), whose apical meristems are attacked by the larvae of *Platyptilia carduidactyla* (artichoke plume moth), during 2012 to determine whether apical meristem mining affects *C. altissimum* fitness and to determine whether these effects arise indirectly through plant-mediated effects on floral visitation. In a restored tall grass prairie, 180 tall thistle adult plants were randomly selected and assigned randomly to treat with insecticide, water and unmanipulated control. On these plants, I quantified effects of apical meristem mining on plant architecture, flowering phenology, flower visitors' activity and seed production.

Apical meristem miners affected several aspects of plant architecture, including reducing plant height and increasing the proportion of axial flower heads, and many aspects of plant flowering phenology, including delaying flowering and date of maximum floral display. Apical meristem miners significantly decreased *C. altissimum* lifetime seed production, showing their strong effects on plant fitness. *Bombus pensylvanicus* and *Melissodes desponsa* were the most common visitors on *C. altissimum* flower heads. No strong effect of apical meristem miners was reported on the behavior of bee (Apidae) species, which may have resulted from the availability of the major visitors of *C. altissimum* flower heads throughout the flowering season. Overall, apical meristem mining strongly affected the plant reproduction success but no evidence was found to suggest that these effects on fitness of *C. altissimum* arose through changes in floral visitation. Being a monocarpic plant with little seed bank, reduced seed production by *C. altissimum* may translate into smaller population sizes.

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CHAPTER 1

INTRODUCTION

1.1 Significance of Herbivores' Effects on Plant Fitness and Mechanisms of these Effects.

When herbivory reduces host plant fitness, a fundamental question is "what are the mechanisms by which herbivores affect plant fitness?" Often ecologists think of negative effects of herbivory on plant fitness as arising from loss of resources or reduced ability to acquire resources by the plant as a result of loss of the tissue that is consumed by the herbivores. However, effects of herbivore taxa or guilds on plant fitness may arise if herbivore damage modifies the plant's interactions with other herbivores, competitors or mutualists. In other words, herbivores may have indirect effects upon plant fitness (Wootton 1994, Parra-Tabla and Herrera 2010). For example, effects of herbivory may be more severe when the host plant is competing against its neighbor plants and the loss of tissue puts the plant at a disadvantage in competitive interactions (Hamback and Beckerman 2003). Also, herbivory can change the community structure through altering the competitive interactions of a dominant plant (Hunt-Joshi et al. 2004).

Insect herbivore exclusion experiments have demonstrated that insect herbivores can reduce host plant fitness (Rausher and Feeny 1980, Louda and Potvin 1995, Freeman et al.2003, Miller et al.2009). Insect herbivores affect plant fitness in various ways including reduction in total number of flowers produced (Brody and Irwin 2012), decreasing seed weight (Benner 1988) and seed number (Huebner 2011, Barber et al. 2012, West 2012), increasing selfing in plants (Penet et al. 2008), affecting ovule fertilization (Romero and Neto 2005), making flowers less attractive for pollinators (Kessler and Halitschke 2009), destroying the floral structures and

seeds (Boieiro et al. 2012) and affecting the host plant fitness greatly through the additive effects of herbivory (Irwin and Brody 2011).

Understanding effects of insect herbivores on host plant fitness is of fundamental significance for the evolutionary biology and ecology of plant-animal interactions as well as being of applied significance for control of noxious weeds. For example, the exceptional diversity of insects and angiosperms has been attributed to their interactions as herbivores and host plants. Ehrlich and Raven (1964) from their studies on butterflies and their host plants hypothesized that escaping insect herbivores allows plant populations to expand and colonize new habitats. Natural selection with the reciprocal evolutionary interactions between herbivores and their host plants can lead them to a long coevolutionary history and diversification (Ehrlich and Raven 1964). Similarly, understanding plant-herbivore interactions has applied significance in the field of agriculture and invasive species management. It helps to test the assumption of classical biological control of weeds that insect herbivores can limit plant population growth (Louda and Potvin 1995). If this assumption is not valid then classical biological control might result in introducing exotic insects with very little hope of achieving control of the weed population.

Only recently have ecologists started to explore the possibility that herbivory affects fitness through altered plant-pollinator (or flower visitor) interactions. Herbivore-induced changes in plant size, architecture, phenology and tissue chemistry all could affect host plant interactions with floral visiting insects. In turn, the number of floral visitors (Krupnick and Weis 1999, Adler et al. 2001, Cardel and Koptur 2010), their behavior (duration of visit to individual flowers, movement within a plant vs. between plants) (Krupnick and Weis 1999) and the species composition of floral visitors (Strauss 1997) could affect host plant seed production and fitness.

But, to what extent are these aspects of the interaction between floral visitors and their host plants influenced by insect herbivore damage to the host plant?

In this study I quantify effects of apical meristem mining insects upon lifetime seed production and seed quality of the short-lived, monocarpic perennial plant, *Cirsium altissimum*. Further, I evaluate the hypothesis that such effects may arise through changes in floral visitation mediated by herbivore-induced changes in host plant traits.

1.2 Effects of Insect Herbivores on Host Plant Fitness

Hairston et al. (1960)'s 'world is green hypothesis', suggests that herbivores (including insects and others) should rarely affect plant performance, including seed production. Similarly, Crawley (1989) suggested that plants can have a more influential role in insect population dynamics than the insects have on plants. However, evidence is accumulating that many different guilds of insect herbivores can limit lifetime seed production by their host plants. For example, florivory significantly limited life time seed production and reduced maternal fitness in *Cirsium canescens* (Potvin and Louda 1995). Florivory by a beetle (*Meligethes rufimanus*) directly and negatively affected male and female reproduction success in *Isomeris arborea* by consuming the gametes (Krupnick and Weis 1999). Increased self-fertilization in *Fragaria virginiana* was found because of florivory (Penet et al.2008). Similarly, Rausher and Feeny (1980) found an increased mortality rate for *Aristolochia reticulata* plants that suffered folivory by the larvae of *Battus philenor* and damaged plants were projected to have <5% of the lifetime seed production of undamaged plants Agrawal (2001) observed caterpillar folivory on different families of greenhouse grown *Raphanus raphanistrum* and found that herbivory reduced the parent plant's fitness with reduced production of seeds and reduced seed viability in some families.

Besides insect herbivores that are highly specific in the plant tissues they attack, there are several specialist and generalist herbivores which eat multiple organs or tissue types of the same host plant. Miller et al. (2009), in an insect exclusion experiment involving a long lived-native cactus (*Opuntia imbricata)* in desert grasslands in New Mexico, found that seed production and plant growth were greatly reduced by the different guilds of insect herbivores (e.g.; A weevil: *Gerstaekeria* species and the cactus beetle: *Moneilema appressum*) which eat every part (vegetative and reproductive parts) of the plants. Further, they showed a strong effect of insect herbivory on population growth of a perennial plant. Herbivory on a parent plant may even affect the fitness of the off-spring. Off-spring produced from damaged (by granivory) parent plants of *Raphanus raphanistrum* showed low vigor and high susceptibility to viral diseases (Agrawal 2001).

Effects of apical meristem mining on host plant fitness are particularly intriguing because this damage may remove little tissue, but may have large effects on resource allocation patterns and interactions with other species. After their apical meristems were damaged by insects, *Cirsium canescens* and *C. undulatum* plants produced less seeds (West 2012) than undamaged plants. Fewer and lighter seeds were developed by *Thlaspi arvense* because of delayed flowering due to apex removal (Benner 1988). About 50 % reductions in total seeds due to apical meristem damage (clipping) was reported in *Ipomopsis aggregata* (Brody and Irwin 2012).

For all plants, seed production is important to maternal fitness. However, for monocarpic perennial plants population size often may be limited by seed availability (Louda and Potvin 1995, Maron and Crone 2006, West 2012). Monocarpic plants have a single episode of sexual reproduction in their lifetime and, therefore, have only one chance to produce seeds. As they rarely have any form of vegetative or asexual reproduction, these plants cannot maintain their

population size during those years when there is a very low seed production. Further, short lived monocarpic perennial plants have to rely more on current seed production for regeneration because they often have a transient seed bank (Louda 1994, Louda and Potvin 1995). In other words, if these plants suffer reduced seed production as a result of insect herbivory (or reduced pollination success by any means) then there is a strong probability that the number of plants recruiting in the next generation will also be lower (Louda 1994, Louda and Potvin 1995, Maron and Crone 2006). In such a seed limited case, the addition of extra seeds can produce larger quantities of seedlings and reproductive adults as shown by Russell et al. (2010) for *C. altissimum* in restored tallgrass prairie in eastern Nebraska.

1.3 Effects of Herbivory on Plant Architecture and Flowering Phenology

Indirect effects of insect herbivory on host plant fitness that arise through changes in floral visitation often may be mediated by changes in traits of the shared host plant. For example, herbivory can change plant architecture. Floral visitors' behavior could be different if the plant's architecture and height are changed after it is damaged by herbivores because architectural changes may affect the apparency of the plant or foraging decisions by floral visitors. Galls formed by moths in *Silphium integrifolium* shoots caused reduced shoot growth, leaf and flower head production (Fay and Harnett 1991). Brody et al. (2007) found that *Ipomopsis aggregata* produced multiple stalked inflorescences after insect and mammalian herbivores damaged the plant's apical meristem whereas undamaged plants' inflorescences consisted of a single stalk.

Herbivores that attack and damage particularly the apical meristem can change the plant height and increase the branching more effectively than other herbivore guilds by releasing

apical dominance. So, damage of the apical meristem can have several negative effects on plants. For example, apical heads produced by plants with an intact apical meristem may be larger and more conspicuous for pollinators than heads produced by plants with damaged a apical meristem (West 2012). Kleunen et al. (2004) found that clipped plants produced smaller inflorescences than unclipped plants. Shoot apex removal increased branching in *Verbascum thapsus* and decreased plant height (Naber and Aarssen 1998). Similarly, apical meristem removal increased the branching in *Thlaspi arvense* plants (Benner 1988) and the clipped plants had lower total number of flowers produced in *Ipomopsis aggregata* (Brody and Irwin 2012).

Damage to vegetative parts, like leaves, stems or roots by insect herbivory, can also reduce floral production (Quesada et al. 1995, Strauss et al. 1996) through changes in flower size and number (Strauss 1997). For example, folivory reduced petal size in *Erigeron glaucus* (English-Loeb and Karban 1992). Similarly, Lehtila and Strauss (1997) found that flower number and size (petal size; length× width) were reduced in *Raphanus raphanistrum* after *Pieris rapae* larvae damaged their leaves, and consequently native bees visited the undamaged plants more often than the damaged ones.

Along with the size of the floral display (number of flowers or inflorescences), the timing of flower production could be affected by herbivory (Rodriguez-Robles et al. 1992, Ohashi and Yahara 2002, Grindeland et al. 2005) influencing floral visitation (Strauss et al. 1996, Strauss 1997, Ohashi and Yahara 1998). From these studies, it seems like flowering phenology can not only be changed by the herbivorous insects but also can affect the different types of herbivores and visitors to a plant. So, the change in flowering phenology of a host plant, after it suffers herbivory, may have a strong effect on plant reproductive success. Early-season leaf herbivory delayed flowering, reduced petal size and lowered pollen production in *Erigeron glaucus*

(English-Loeb and Karban 1992). Leaf herbivory by *Pieris rapae* delayed the initial date of flowering in *Sinapis arvensis* (Poveda et al. 2003). Similarly, galls formed by moths in *Silphium integrifolium* shoots caused delayed flowering (Fay and Harnett 1991). Floral herbivory, by delaying flowering phenology, can alter the synchrony of flower production and pollinator activity, which is likely to have a significant effect on plant fitness (English-Loeb and Karban 1992, Krupnick and Weis 1999).

Shorter flowering duration provides less opportunity for floral visitation, which could increase the likelihood that the quantity of seed produced, will be limited by pollen availability. Louda and Potvin (1995) noted that inflorescence feeding insects even shortened the duration of flowering time in *Cirsium canescens,* which ultimately resulted in fewer flowers available to pollinators. However, despite its importance, no study was found focusing particularly upon the effects of insect herbivory on flowering duration of its host plant.

It is apparent that most studies dealing with apical meristem damage involve simulated damage, like clipping the apex manually, rather than manipulating actual apical meristem damage by herbivores. Being a main growth part of the plant body, damage of the apex may have most influential effects on plant fitness. Further, simulated herbivory may not always represent the actual herbivory properly because of the unique nature, timing and extent of damage by the herbivores. So, the current study attempts to manipulate apical meristem damage by the actual herbivores.

1.4 Effects of Herbivory on the Activities of Floral Visitors

Changes in plants' size and architecture (like plant height, branching, floral display, size of inflorescence etc.) due to herbivory may affect floral visitors/pollinators in diverse ways.

Changes in inflorescence size that occur as a result of herbivore damage may represent particularly important architectural changes for influencing floral visitor behavior. Whether the inflorescence is altered by herbivory or develops naturally, Wyatt (1982), by comparing different types of determinate and indeterminate inflorescences across plant species, found that inflorescence architecture affects pollen movement, quantity of fruits matured and seed weight. Changes in inflorescence architecture can directly alter the interactions between damaged plants and pollinators affecting the pollen movement (Wyatt 1982, Juenger and Bergelson 1997). For example, movement of pollinators could be shifted from among flowers on different plants to among flowers of the same plant, changing rates of out-crossing vs. self-fertilization through geitonogamy (Ishii and Harder 2006). Since the off-spring that result from self-pollination often may be less vigorous than off-spring that result from out-crossing, changes in the ratio of self-fertilized to out-crossed off-spring can have a negative impact on the host plants' fitness. Plants displaying many and large flowers or inflorescence attract more pollinators than plants with fewer or smaller flowers (Herrera and Pellmyr 2002). While studying interactions between herbivores (flower and fruit) and pollinators of a perennial herb *Helleborous foetidus*, Herrera et al. (2002) found that pollinators preferred larger floral displays. However, large floral displays also present a disadvantage in that they increase geitonogamy when the pollinators tend to visit more flowers on the same plant, potentially increasing self-pollination (Ishii and Harder 2006).

Changes in flower size and number as a result of foliar herbivory can affect the attractiveness of plant floral displays to pollinators (Strauss 1997). Bees visited larger floral displays in *Cirsium purpuratum* (Ohashi and Yahara 1998, 2002). Strauss et al. (1996) demonstrated that pollinators visited flowers of *Raphanus raphanistrum* plants whose leaves had been damaged by butterfly larvae (*Pieris rapae*) less than undamaged plants and spent less time

on the damaged plants during each visit. Plants with damaged leaves produced flowers with smaller petals (smaller in length and width) apparently affecting the flowers' attractiveness to pollinators. Thus changes in floral attraction and reward characters as a result of herbivory could alter both the numbers and the behavior of pollinators.

Changes in host plants that result from herbivore damage may influence species composition of floral visitors, in addition to altering the frequency of visitation and visitor behavior. Different taxonomic groups of floral visitors may be most strongly attracted by different aspects of the floral display, providing a mechanism by which floral visitors may not be affected uniformly by herbivore damage to host plants. For example, syrphid flies' visitation on *Raphanus raphanistrum* was most affected by changes in petal length whereas small bees' visitation was most affected by changes in number of flowers open (Strauss et al. 1996, Lehtila and Strauss 1997). These changes in species composition of flower visitors/pollinators could affect seed production, since different species of pollinators may not be equally effective in transferring pollen.

1.5 Research Questions

To quantify effects of apical meristem mining upon host plant lifetime seed production and to examine the possibility that such effects might arise indirectly through effects on floral visitation, I used as a study system the native, monocarpic perennial tall thistle (*Cirsium altissimum*), and its suite of floral visiting insects. Specifically, I addressed the following research questions:

1. Does apical meristem mining affect lifetime seed production, seed quality and the vigor of seedlings produced by *C. altissimum*?

2. Does apical meristem mining by insects alter the architecture and flowering phenology of *C. altissimum*?

3. Does apical meristem mining alter the species composition of insect floral visitors to *C. altissimum*?

4. How do floral visitors belonging to the bee family Apidae change their behavior on *C. altissimum*, including frequency and length of visits and patterns of within plant movement, on tall thistle plants that have suffered apical meristem mining?

CHAPTER 2

MATERIALS AND METHODS

2.1 Study Species

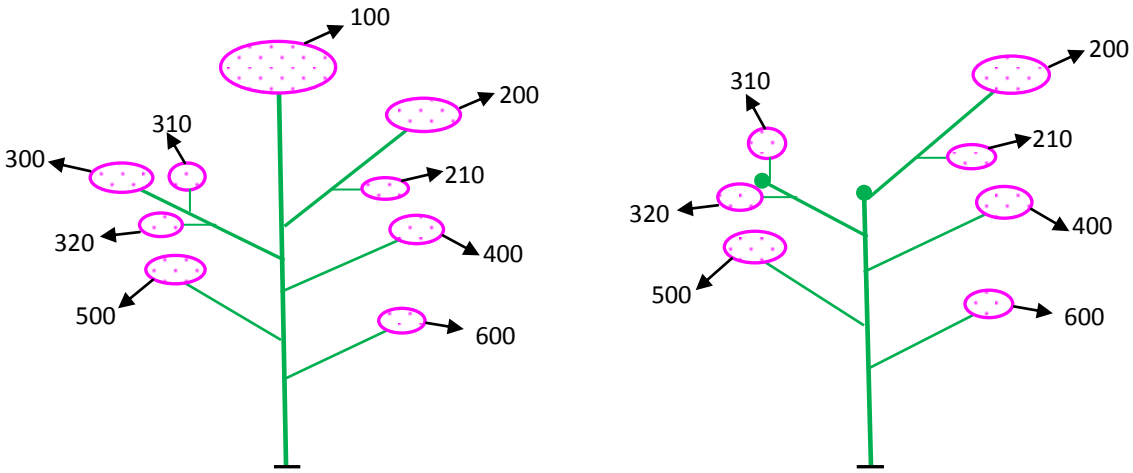
Cirsium altissimum L. Spreng.(Tall thistle), a native North American species, is a short-lived perennial, monocarpic plant that occurs in small to big patches in roadsides, ditches, pastures and moderately disturbed non-cultivated sites or wastelands. Adults are 1-2.5 m tall having branched stems. Leaves are long (10-30 cm), green, glabrous and serrated. Flower heads are spiny and solitary or terminal with involucres 2-3.5 cm tall and 2-3.8 cm wide. This plant has mostly dark purple or lighter corollas that are 22-32 mm long. Its achenes are pale brownish (4.5-6 mm long) and its pappus (17-27 mm long) is white or grayish (Great Plains Flora Association 1986).

Cirsium altissimum occurs throughout the eastern United States as far west as central Kansas. It is the most common thistle in tall grass prairie of the eastern Great Plains including central Kansas (Great Plains Flora Association 1986). Generally, adult *C. altissimum* plants begin producing a reproductive stalk in early May, flower in late July-October and disperse seeds in September –early November (*Pers. Obs.*).

C. altissimum is attacked by a diversity of insect herbivore guilds. Commonly reported folivores of *C. altissimum* include the native weevil *Baris subsimilis*, the exotic weevil *Trichosirocalus horridus* (Takahashi et al. 2009), larvae of the painted lady butterfly *Vanessa cardui*, a flea beetle *Systena hudsonias* (Russell et al. 2010), and grasshoppers and several microlepidopterans (Guretzky and Louda 1997). On reproductive tissues, the main insect herbivores are the meristem-mining moth *Platyptilia carduidactyla*, and two species whose

larvae attack developing flower heads *Paracantha culta* (a tephritid fly) and *Homoeosoma eremophasma* (a moth) (Rose et al. 2011).

Research in Nebraska has shown that insect herbivory on *C. altissimum* is common (Guretzky and Louda 1997, Takahashi et al. 2009) and strongly affects plant survival at juvenile stages (Russell et al. 2010). In addition, insect herbivory on *C. altissimum* damages flower heads and apical meristems significantly. For tall thistle populations in southeast Nebraska, the combined effect of all insect herbivore guilds is to reduce tall thistle maternal fitness as well as population growth rates (Rose et al. 2011).



A. Plant with intact apical meristem B. Plant with mined apical meristem

Figure 1. Diagrammatic representation of tall thistle plant showing the numbering for each head produced (*In the text below, the 100 head is termed as apical head, all the first order heads like 200, 300, 400 and so on are termed as lateral heads and the secondary heads like 210, 310, 320 are termed as axial heads*).

To identify individual flower heads on adult tall thistles, I use a numbering system developed by Dr. Svata Louda, University of Nebraska-Lincoln. Flower head numbers start with the apical flower head, which I refer to as '100.' Moving basally from the 100 flower head, terminal heads on lateral branches are referred to as 200, 300 and so on (Figure 1 A & B). Axial

heads produced on branchlets of those lateral branches were numbered as 210, 220, 230 or 310, 320 and so on.

2.2 Study Site

The two study sites for this research were located at Wichita State University's Ninnescah Reserve (37.32^0 N, 97.40^0 W), near Viola, Kansas USA. The habitat at the Ninnescah Reserve is restored tallgrass prairie that was converted from agriculture 30 years ago. These restored prairies are dominated by warm season grasses, including *Andropogon gerardii* (big bluestem), *Schizachyrium scoparium* (little bluestem), *Sorghastrum nutans* (Indian grass), *Panicum virgatum* (switch grass), and forbs such as *Helianthus maximiliani* (maximilian sunflower) and *Cirsium altissimum* (tall thistle), with moderate invasion by shrubs, principally coralberry (*Symphoricarpos orbiculatus*) (*Pers. Obs.*). The areas have been heavily invaded by the exotic species, *Bromus tectorum* (cheat grass).

Two sites (hereafter A and B) were selected for this study after a brief survey of the reserve to identify locations where a sufficient number of tall thistles were present. Site A lies approximately 100 m east and site B lies approximately 10 m west of the hedgerow along Rd 295. The sites have not been grazed since establishment of the reserve (35 years). Site A was not mowed for several years before my study began so it was extensively invaded by woody vegetation whereas site B has been mowed regularly, including in 2011, so it was rich in grasses with little woody vegetation and some poison ivy (*Toxicodendron radicans*). Soils at both sites were dry sandy loam with site A richer in humus than B (*Pers. Obs*). Both sites are within the 100 year floodplain of the Ninnescah River.

I further characterized the two sites as part of my field work. Soil moisture was measured in June and October (2012) 50 cm north of each sampling point. Sampling points were randomly selected near the experimental plants on each site. There were 21 points in June and 25 in October. Soil moisture was measured using a FieldScout TDR 100 Soil Moisture Meter (Spectrum Technologies, Inc., Illinois, USA) with 12 cm probes. To quantify aboveground plant biomass, two long transects (along a north-south axis) were established in both sites and 10 points at intervals of 10 m were selected. Then, a 25 cm*25 cm quadrat was placed at the sampling point and all plants inside the quadrat were clipped at the soil surface, sorted to species, dried and weighed. The dry biomass (for the total and woody vegetation only) was used to analyze the community productivity of the study area. Species composition was also described using Shannon's diversity index, Pielou's evenness index and Bray-Curtis similarity index.

2.3 Experimental Design

2.3.1 Establishing the Experiment

Because *C. altissimum* rosettes begin producing a reproductive stalk in early May (FL Russell and S Adhikari, *Pers. Obs.*), I began field work in April 2012 in order to control apical meristem miners who can damage meristematic tissue at the center of the rosette before the plant starts to bolt. To select naturally-occurring adult tall thistle plants for my experiment, I established transects in both sites (112.5 m in site A and 107.5 m in site B) along the longest axis through the tall thistle population. At 2.5 m intervals along the transects, the two nearest adult tall thistles with the apical meristem undamaged were selected with the constraint that experimental plants had to be >2 m apart. As far as possible, very big and very small plants were avoided to maintain an approximate uniformity among selected plants.

Each plant was flagged and tagged with an identifying number. I randomly assigned treatment levels to each plant by tossing a coin. First, insecticide and control plants were assigned. Second, the control plants were further divided into water and unmanipulated control. This procedure resulted in 46 insecticide, 23 water control and 23 unmanipulated control plants in site A and 44 insecticide, 22 water and 22 unmanipulated control plants in site B. Only plants with intact apical meristems were selected for the study.

Bifen I/T (Control Solutions, Inc. Pasadena, TX, USA), a non-systemic synthetic pyrethroid insecticide, was mixed in 1:15 ratio with water and applied only on the apical meristems of the 90 plants assigned to the insecticide treatment by using a small-pointed, hand-held sprayer. Insecticide application was stopped when the apical flower head started to appear. For the 45 water treatment plants, a different sprayer of the same type was used to spray water only on the apical meristems. Water application was stopped at the same time when insecticide application was stopped. The remaining 45 plants were left unmanipulated. Root crown diameter was measured just below the shoot-root junction of each plant using a vernier caliper. Height at the beginning of the growing season was measured at the apex of the apical meristem and the rosette diameter was measured across any two longest, opposite leaves of each tagged plant.

2.3.2 Effects of Apical Meristem Mining on Architecture and Flowering Phenology

To quantify architecture of adult tall thistles in the experiment, the height (distance from the ground to the top of highest flower head on the plant) of each plant was measured. For each plant, I also counted the number of first order lateral branches, total number of flower heads and number of flower heads that were blooming. All of these architectural measurements were taken when the most apical flower head of the plant was first observed in full bloom.

To quantify effects of apical meristem mining on tall thistle flowering phenology, I observed all tagged plants each week from early July through the end of flowering in fall 2012 (October 28). On each census, I recorded the number of flower heads (including small pre-anthesis flower heads to mature post-anthesis flower heads) present on each plant along with each flower head's developmental stage. The developmental stages that I used for flower heads were: 1= small bud (tight, unopened bud), 2= large bud (developing bud), 3=early flowering (flowers open but stigmas not exerted), 4=late flowering (flowers open and stigmas exerted), 5= mature (flower wilting), and 6= dispersing (Adhikari 2003). Diameter of each flower head on each experimental plant was recorded when it was in full blooming stage (i.e. condition 3 or 4).

2.3.3 Observation of Floral Visitors' Activities

I focused my collecting efforts on bees, which are important pollinators in *Cirsium* spp. (Ohashi and Yahara 1998, Jump et al. 2003, Theis and Raguso 2005, *Pers. Obs*. 2011). To identify bees that visit tall thistle flower heads to the lowest possible taxonomic level, I collected insect floral visitors from naturally-occurring tall thistle plants near sites A and B. Insects were not collected from the experimental plants. Any insect found foraging on flower heads of tall thistle plants was collected for 7 weeks (every week from the second week of August to the end of September, 2012). This time interval corresponded to peak blooming in the tall thistle population. More than 10 hours in total was invested in collecting insects from tall thistle flower heads. I made collections any time between 7 am and 6 pm. Insects were trapped by using a trapping net and put in a killing jar. Insects were kept in a freezer and then spread on a spreading board. Finally, they were pinned to create voucher specimens. Microscope, web resources, books and posters were used to identify the bees. My identifications were later confirmed or re-

identified by Drs. Mary Liz Jameson (Wichita State University), Charles Michener (University of Kansas), Terry Griswold (USDA) and Jeff Lozier (The University of Alabama).

I randomly selected 60 of the 180 experimental plants for conducting observations of floral visitation by insects. These 60 plants included 30 plants from the insecticide treatment (15 in site A and 15 in site B), 15 from the water-control treatment (8 in site A and 7 in site B) and 15 from the unmanipulated control treatment (7 in site A and 8 in site B). I began observing insect floral visitors on a selected plant when it first had at least one flower head blooming. After I found bees (mainly the Apidae) to be the most common visitors of tall thistle (*Pers. Obs* in 2011 & 2012), I focused my observations only on the activities of bee visitors on the thistle flower heads.

Because very early morning and late evening were likely to have little insect activity (Rodriguez-Robles et al.1992), I observed floral visitors' activities between 8 am and 4 pm. As I could not observe floral visitors on all 60 selected plants in a single day, I randomly selected a sub-set of 30 plants (15 insecticide treatment, 8 water treatment and 7 control or 7 water treatment and 8 control) from across both sites among the currently blooming plants for observation. This balanced representation of treatments in a single day's observations avoided confounding treatment with different weather conditions on different days.

To collect data on the taxonomic composition of visiting insects and, for bees, their visitation rates (number of visits per unit time), time spent on a flower head during an individual visit and movement among flower heads within a plant, I stood quietly near the plant that I was observing and recorded the visitor's activity by using timers with lap systems to separate the time spent by different individual bees. In addition to collecting data on the insects, I noted the weather conditions (sunny, cloudy, rainy, semi-cloudy and sunny or no sun-no rain-no cloud),

time (morning, afternoon, evening) and the size of the flowering display on the plant (total number of flower heads and number of heads flowering).

Whether there were floral visitors or not, I spent 12 minutes (Lehtila and Strauss 1997) observing each selected plant. I divided the 12 minute observation period into two 6 minute segments. The first 6 minutes were spent observing a single flower head and recording (focusing only on Apidae) the identity of visitors to the lowest taxonomic level possible, how many visitors visited flower heads and how much time each visitor spent on the flower head. Any available flower head starting from the top that was in peak blooming (i.e. the flower heads with condition 3 or 4 with all florets exerted exposing the anthers clearly to the visitors and remaining purple) was selected for watching the flower visitors. During each observation period, I recorded the identity of the flower head (flower head number in Fig. 1) on which the observations were made. The second 6 minute segment was spent observing movements among flower heads on the same plant by individual bees in the Apidae if there was more than one flower head blooming. I recorded data on intra-plant movements for the first Apidae individual that arrived at any blooming flower head of the selected plant. The movement was observed to assess whether apical meristem damage affects the likelihood of geitonogamy. In both 6 minute segments, I did not spend more time than allocated even if a visitor remained on the plant at the end of my prescheduled 6 minutes time. If there was only one flower head blooming per plant (which was very common), I recorded the visitation rates and time spent on the single flower head for the whole 12 minutes.

2.3.4 Quantification of Seed Production

To quantify the effect of apical meristem mining on seed production by *C. altissimum*, the remaining 120 experimental plants, after 60 plants were selected for floral visitor observation, were used. Different plants were used for observing insect behavior and for quantifying seed production to avoid any possible influence of the presence of flower head bags (for catching seeds before they disperse) on insect visitation and behavior on the unbagged flowering heads. Bags made of organza fabric were placed over post-anthesis flower heads to catch the seeds. I bagged all post-anthesis flower heads on each selected plant. Bagged heads were collected after seeds began dispersing into the bag. I dissected the collected flower heads in the lab to determine the number of viable seeds produced by each flower head. All filled, undamaged seeds were considered as viable seeds. Seeds were considered to be 'filled' if the sides of the seed were convex. For a monocarpic plant, total viable seed produced by all flower heads on a plant during its one reproductive episode represents an estimate of maternal fitness. The viable seeds produced by each flower head were weighed to determine if there was any difference in mass among seeds produced by different plants in different treatment levels and also by different flower head positions.

2.3.5 Seed Germination and Seedling Biomass

To compare the quality of seeds produced by plants under different treatment levels, undamaged, viable seeds from a sub-set of flower heads collected from the experimental plants were sown in a greenhouse. Their germination rates and seedling biomass were recorded. This greenhouse experiment also allowed me to compare seed quality among flower heads in different positions on the tall thistle plant (mainly the apical heads with the other heads).

For this seed germination and seedling biomass study, only heads that produced ≥30 viable seeds were selected. All of the 100 (apical) flower heads were selected if the plant produced a lower terminal flower head on a lateral branch for comparison with the apical head. If there was no 100 head from an experimental plant then the most apical and the most basal available terminal heads on lateral branches heads were selected. Axial heads (e.g. 310 or 320) were selected only if the terminal flower head on the same lateral branch (e.g. 300) was available for comparison. If more than one axial head was present on a lateral branch then the most basal head was selected. Insecticide treatment plants without 100 heads were not selected. Coincidentally, only heads collected after Sept 15 and before Oct 14 met the criteria for selecting flower heads. The first date of collection was Sept 15 and the last date was Oct 28, 2012. Out of 321 flower heads (from 86 different plants) dissected, only 127 flower heads (94 from insecticide plants, 13 from water-control plants, and 20 from unmanipulated control plants) fit the criteria for sowing the seeds to assess seed and seedling quality. These heads were from 51 plants (36 insecticide, 6 water, and 9 unmanipulated controls). 30 seeds from each selected head were sown.

Fresh soil was collected from Wichita State University's Gerber Reserve (37.68°N, 97.95°W). While collecting the soil, areas where tall thistles had been observed in the previous 2 years were avoided. Soils from different buckets, which represent different collection locations at the Gerber Reserve and different depths of collection, were mixed together to make a homogenized soil in all pots. Labeled square pots (Volume: 1.78 L; Inside diameter =12.54 cm, height=14.29 cm) were filled with soil and 30 seeds of any single flower head were sown in each pot on December 25, 2012. Pots were watered regularly as needed and ambient light was augmented for 12 hours a day with a 1000 Watt halogen bulb. To avoid the differential

environmental effects (direction of light source, edges of the benches etc.) on different pots, all pots were rotated clockwise every Monday. In total 6 such rotations were conducted.

Proportion of seeds germinating was quantified for each pot (flower head). Seedlings were recorded as having germinated once their two cotyledons were clearly visible. Only the first four seedlings to germinate in each pot, which I labeled with colored pins, were kept to quantify seedling biomass, all other seedlings were pulled upon producing two cotyledons. The germination rates (the proportion of seeds that germinated among the 30 seeds sown) were calculated. Five weeks after seed sowing or 4 weeks after the mean germination date for the first four seedlings, all 4 seedlings in a pot were harvested to determine the total dry tall thistle biomass for each pot and so for each flower head. All seedlings in a pot were harvested simultaneously to allow them growing for the same time period (average 4 weeks per each pot) from their germination date. So, because of the differential germination rates, it took 11 days (Feb 1-10) to harvest seedlings of all pots. Each seedling was divided into aboveground and belowground plant parts; they were dried and weighed to quantify aboveground, belowground and total dry biomass. The resulting germination rates and dry biomass of the offspring from plants under different treatment levels provided information about seed viability and seedling vigor.

2.4 Self-Compatibility Study

If a plant is completely self-compatible then changes in amount of floral visitation received will have no impact on seed production because the plant will produce seeds through selfing. Similarly, off-spring produced by cross pollination often have greater fitness than off-spring produced by selfing (Byers 1998, Galloway et al. 2003). So, to evaluate whether tall thistle is

self-compatible, I conducted a pollinator exclusion experiment at the Ninnescah Reserve in September-October 2011.

On Sept 7 2011, I selected the first 20 tall thistle adults that I encountered that had two un-opened flower heads of similar size and similar developmental stages. One unopened head on each plant was randomly assigned to be bagged with organza fabric to prevent pollen transfer and the other unopened head was left unbagged and, therefore, open to pollinator visitation as a control. Unless bags ripped, any seed produced by the bagged head will be the result of self-pollination. Once the flower heads had finished blooming (stage 5), bags were added to 'open pollination control' heads on experimental plants to prevent any loss of seeds (pollinator exclusion heads were already bagged). After flower heads began dispersing into bags, they were collected on Oct 14 2011.

In the lab, diameters of flower heads were measured and the heads were dissected to quantify seed production. I counted seeds in different combinations of developmental and damage categories. Developmental categories were "shriveled," "unfilled," and "filled." Damage categories were "damaged" or "undamaged." Seeds that were "filled," as indicated by their convex sides, were considered to be viable.

2.5 Statistical Analyses

For each dependent variable to be analyzed, I first attempted parametric statistical analyses. Parametric analyses are presented only after the assumption was met for the test of normality of residuals (Kolmogorov Smirnov test) and for the equality of variance in different samples (Levene's test). In case those assumptions of parametric tests were not met, non-parametric analyses (Kruskal-Wallis test) were performed and are reported.

For each parametric ANOVA, non-parametric test and contingency table analysis, water treatment (W) and unmanipulated control (N) were compared first. If they were found similar then they were pooled to make 'control'(C) and compared with insecticide (I) treatment plants. Otherwise, water and unmanipulated control were compared separately with insecticide treatment plants. In ANOVAs that analyzed effects of the experimental treatment, site was treated as a random effect. For non-parametric and contingency table analyses, at first the two sites were compared. If there was no significant difference between the sites then observations from the two sites were pooled. Otherwise, they were treated separately. When the sites were treated separately, I applied a Bonferroni correction to the significance threshold, setting the alpha value (p) at 0.025 (instead of 0.05 when combined together). All statistical analyses were conducted using SAS version 9.1 (SAS Institute, North Carolina, USA).

Site Characteristics

Mean aboveground plant community biomass, mean soil moisture (in June and October), and plant species richness were compared between sites A and B by one way ANOVA. Mean aboveground woody plant biomass was analyzed with a Kruskal-Wallis test because it did not meet the ANOVA assumption for normality of residuals.

Effects of Insecticide Application on Insect Herbivore Damage

To assess whether insecticide application was effective in preventing apical meristem mining, the frequency of apical meristem damage in experimental plants was analyzed with a contingency table. To determine whether the insecticide application had unintended effects on damage by other guilds of insect herbivores on tall thistle, proportion of flower heads damaged

per plant was analyzed with chi-square analysis of a two way contingency table and proportion of leaf damaged per plant was analyzed with an ANOVA.

Plant Fitness

Treatment effects on mortality rates of experimental plants were analyzed by using a chi-square analysis of a two way contingency table.

Total number of viable seeds produced by each experimental plant and the total mass of seeds per each plant were analyzed with Kruskal-Wallis tests. These analyses were performed for a data set that included all experimental plants, including those that died before flowering, and for a data set that included only those experimental plants that survived to flower.

The effects of the experimental treatment and flower head position within a plant on germination rate of seeds (taken from the selected flower heads of the experimental plants) and seedling biomass were analyzed with Kruskal-Wallis tests.

Plant Architecture and Flowering Phenology

For plant architecture, morphological variables, including plant height, number of branches, branch density (number of branches per unit stem height), number of leaves, number of total flower heads, total flower head density (number of flower heads per branch), number of flower heads that bloomed, and proportion of axial flower heads that bloomed, were analyzed with Kruskal-Wallis tests. Phenological variables, including initial date of flowering, flowering duration, maximum floral display and the date with maximum number of flower heads blooming, were analyzed by using chi-square analyses of two way contingency tables. These phenological

variables were treated as categorical, rather than continuous, because they had a narrow range of values.

Floral Visitation

For the floral visitation data, including length of visits for Apidae individuals, total time spent by all Apidae, total number of Apidae visiting per minute and total taxa visiting flower per minute, I used Kruskal-Wallis tests for the analysis. Logistic regression was used to analyze treatment effects on the probability that the flower head I was observing would be visited by Apidae.

Self-compatibility

Plant self-compatibility was tested by comparing production of total seeds, filled seeds and shriveled seeds between the paired bagged and unbagged flower heads on each experimental plant using a paired t-test.

CHAPTER 3

RESULTS

3.1 Comparison of Study Site Characteristics

Average soil moisture, measured as % water by volume, at site A (3.24%±0.34) taken June 16, 2012 was not significantly ($F_{1,40}$=2.03, p=0.16) different than at site B (2.58%±0.29). However, soil moisture on October 7, 2012 was marginally significantly ($F_{1,48}$=3.2, p=0.079) lower in site A (5.38%±0.54) than in site B (6.69%±0.51). Total plant biomass was found to be significantly (Fig. 2; $F_{1,18}$=4.83, p=0.041) higher in site A. Woody plant biomass was marginally significantly higher (χ^2=3.16, df=1, P=0.075) in site A (69.83g±26.12) than in site B (1.7g±1.3).

Plant species richness was not significantly different between the two sites ($F_{1,18}$=0.94, p=0.35). In site A, 23 plants species were recorded with 1.52 Shannon's diversity index and 0.48 Pielou's evenness index. In site B, 17 plant species were found with 1.69 Shannon's diversity index and 0.60 Pielou's evenness index. Bray-Curtis similarity index between the two sites was found as 0.89.

3.2 Effects of Insecticide Application on Insect Herbivore Damage

Application of insecticide to the apical meristem was successful in protecting apical meristems from mining and apparently did not affect damage by other guilds of insect herbivores on tall thistle. The apical meristem on 4.5% of insecticide treatment plants was damaged before producing an apical (100 position) flower head whereas the apical meristem on 78.7% of control plants was damaged (Fig. 3; χ^2=55.84, df =1, P=<.0001. Among those few control plants with

undamaged apical meristems, only 10.5% (2 plants) were from site A suggesting that this site is more prone to apical meristem miner's attack.

Proportion (percentage) of leaves damaged per plant was not significantly different among the treatment levels ($F_{2,2}$=0.74, P=0.57). Proportion of flower heads damaged per plant was also not significantly different between insecticide treatment and control plants (χ^2=3.86, df =2, P=0.145). Only 26.7% of all plants from which flower heads were collected, had their flower heads damaged by the insects.

3.3 Effects of Apical Meristem Mining on Host Plant Fitness

The mortality rate of the insecticide-treated plants was significantly lower (Fig. 4; χ^2=6.38, df =2, P=0.04) than for controls (both water treatment and unmanipulated controls combined).

In total 37,906 filled (viable) seeds were counted from 321 flower heads of 86 plants. Average number of seeds produced by each flower head (for all 321 flower heads) was 118.09±4.37 whereas it was 172.73±9.8 for only the 100 flower heads. Also, average seed mass for all flower heads was 0.68 gm±0.03 and for 100 head was 0.97 gm±0.06.

Including experimental plants that died before flowering and, therefore, produced no seed, total number of seeds produced by the insecticide treatment plants were significantly higher than the seeds produced by control plants in both sites (Fig. 5; Site A- χ^2=9.33, df =1, P=0.0022; Site B- χ^2=19.07, df=1, P=<0.0001) .Total seed mass for each plant was also significantly higher in insecticide plants than in control plants in both sites (Fig. 6; Site A: χ^2= 10.56, df=1, P=0.0012; Site B: χ^2= 19.43, df=1, P=0.0001). Among only those experimental plants that survived to flower, insecticide plants still produced significantly more seeds in site B than the

control plants (Fig. 7; χ^2=5.85, df =1, P=0.001). However, this difference was not significant (χ^2=3.38, df =1, P=0.065) in site A. Similarly, seed mass was also significantly greater in insecticide plants than in control plants in site B (Fig.8; χ^2=6.15, df =1, P=0.001), but only marginally significantly greater (χ^2=4.43, df =1, P=0.035) in site A.

Germination rate of seeds (χ^2=2.437, df =3, P=0.486) and average seedling biomass four weeks after cotyledon emergence (χ^2=5.875, df =3, P=0.117) did not differ between terminal vs. axial flower heads on the same branch or between more apical and more basal terminal heads on a plant selected for the greenhouse experiment. Surprisingly however, average seedling biomass was slightly higher in the control plants than in the insecticide treatment plants even though the difference was only marginally significant (χ^2=3.02, df =1, P=0.082).

3.4 Effects of Apical Meristem Mining on Plant Architecture and Flowering Phenology

3.4.1 Effects of Apical Meristem Mining on Plant Architecture

For final height, plants from site B were significantly taller than plants from site A (Fig. 9; χ^2=12.81, df=1, P=0.0003; A=66.74 cm ±1.53, B=77.16 cm ±1.41). Control plants were significantly shorter (Site A= χ^2=12.05, df=1, P=0.0005; Site B= χ^2=6.45, df=1, P=0.011) than the insecticide plants in both sites.

There was no significant difference in number of flower heads produced between water treatment plants and insecticide treatment plants (**χ^2**= 0.89, df=1, P=0.34; W= 9.41 ±2.04, I=8.51 ±0.70), suggesting no effect of apical meristem mining. However, unmanipulated control plants produced significantly more flower heads (10.66 ±1.21) than the water treatment plants (χ^2= 4.14, df=1, P=0.0419), and also marginally significantly more than the insecticide treatment plants (χ^2= 3.78, df=1, P=0.052). Proportion of flower heads per plant that were axial, rather than

terminal (at the tip of a lateral branch), was significantly higher in control plants than in insecticide plants (Fig. 10; χ^2=6.63, df=1, P=0.01). Number of flower heads that bloomed per plant was significantly higher in site A (χ^2=13.91, df=1, P=0.0002) than in site B. However, unlike results for the total number of flower heads per plant, the number of flower heads that bloomed per plant was marginally significantly higher (χ^2=4.56, df=1, P=0.03 in A and χ^2=3.7, df=1, P=0.05 in B) in insecticide treatment plants than in water treatment plants in site B (I=2.84±0.19, W=2±0.26) but not in site A (I=5.35±0.58, W=4.18±0.99). Similarly, density of total flower heads (heads per branch) was marginally significantly higher (χ^2=3.7, df=1, P=0.054) in insecticide plants than in control (I=1.82±0.06, C=1.84±0.12) plants.

Unmanipulated control plants produced significantly more branches than both water (χ^2= 7.04, df=1, P=0.0080; N=6.31 ±0.72, W=4.30 ±0.41) and insecticide (χ^2= 9.63, df=1, P=0.0019; I=4.52 ±0.36) plants (Table 1). However, water treatment plants did not produce significantly different branches than the insecticide treatment plants (χ^2= 0.045, df=1, P=0.83), suggesting no effect of apical meristem mining on number of branches per plant. There was a trend toward plants at Site A producing more branches than plants at site B (χ^2= 2.76, df=1, P=0.0966; A=5.97 ±0.44, B=3.76 ±0.31). Even though, branch density (number of branches per unit plant height) was not significantly different (χ^2= 0.03, df=1, P=0.85) between water and insecticide plants in site A (Fig. 11; I=0.078±0.007, W=0.078±0.008), it was significantly higher in control plants in site B than in insecticide plants (Fig. 12; χ^2= 14.16, df=1, P=0.0.0002).

Total number of leaves produced by the experimental plants were not different in any treatment levels (W vs. N: χ^2=0.19, df=1, P=0.65; W=48.67 ±6.73, N=47.43 ±6.28 and I vs. C: χ^2=0.399, df=1, P=0.52; I=37.8±1.49, C=48.06±4.58) and in any sites (χ^2=0.27, df=1, P=0.60; A=44.75±3.89, B=41.02±2.88).

The outcomes of all analyses of treatment effects on tall thistle plant architecture are summarized in Table 1.

3.4.2 Effects of Apical Meristem Mining on Flowering Phenology

August 13 was the first date when an experimental plant flowered and the last date on which an experimental plant was flowering was October 21. Interestingly, September 8 was the date when the maximum floral display by an individual plant was observed in both insecticide (5 flower heads blooming on a plant) and in control (7 flower heads were blooming on a plant) experimental plants. However, many experimental plants never had more than one flower head blooming at a time in both insecticide and control plants. Mean flowering duration for the insecticide plants was 2.7 weeks (3.4 in site A and 2.07 in site B) but it was only 1.87 weeks (2 in site A and 1.69 in site B) in control plants.

Apical meristem mining strongly affected the initial date of flowering (the date on which a plant first had a blooming flower head) with insecticide treated plants flowering earlier than control plants (Fig. 13; χ^2=7.19, df=2, P=0.02). Almost 87% of insecticide plants began flowering before September 1 (with 71.2% beginning before 19 August) whereas only 58% of control plants began flowering before September 1 (with only 27.8% beginning before 19 August).

Flowering duration was significantly longer in insecticide treatment plants than in control plants (Fig. 14; χ^2=13.90, df=2, P=0.001). For insecticide treated plants the modal flowering duration was 2 weeks with a maximum of 10 weeks whereas for the control plants the modal flowering duration was 1 week with a maximum of 5 weeks.

There was no significant difference between insecticide treatment and water treatment plants in maximum floral display size (number of flower heads blooming simultaneously). However, unmanipulated plants showed a significant difference from the other two treatment levels (Fig. 15; N vs. W= χ^2=6.71, df=2, P=0.034; N vs. I= χ^2=9.89, df=2, P=0.007)

Peak flowering date (the date on which the largest number of flower heads per plant were blooming) was significantly earlier in insecticide treatment plants than in control plants (Fig. 16; χ^2=5.75, df=1, P=0.01). 64.3% of insecticide plants reached their peak flowering before August 26 whereas only 43.5% of control plants reached peak flowering before August 26. However, peak floral display by the population of experimental plants (total flower heads of all experimental plants blooming in a day) for insecticide plants was on Sept 1 with 90 flower heads blooming on that day and for control plants was Sept 8 with 65 flower heads blooming (Fig. 17).

3.3 Effects of Apical Meristem Mining on the Activities of Floral Visitors

More than 10 hours was spent collecting (1-1.30 hours per week for 7 weeks) floral visiting bees from tall thistle flower heads with almost the same amount of time spent at each of the two sites. However, out of 128 specimens collected, which included 11 bee species, 96 specimens and 11 species were from site B and only 32 specimens and 4 species were from site A (Table 2). The most common visitors to tall thistle flower heads were *Melissodes desponsa* (55/128 specimens) and *Bombus pensylvanicus* (38/128 specimens) (Table 2). Individuals belonging to species in the Apidae were 92.8% of all bees collected and these two common species were 72.7% of all Apidae collected. Also, these two species were collected throughout the peak flowering season (from 2nd week of August to the last of September) and at various times of day. *Agapostemon* sp., *B. fraternus*, *Halictus ligatus*, *Megachile* sp., and *Triepeolus* sp.

were found only during the beginning (2nd week of August) of the flowering season whereas *Anthophora walshii*, *B. fervidus* and *B. griseocollis* were found only during the end (last week of September) of the flowering season (Table 2).

Total time spent for the observation of thistle flower visitors was 11 hours (39,600 seconds). The total time that insects of all taxa (including idle beetles) were present on tall thistle flower heads during observation periods was only 2844 seconds. Bees in the Apidae were present for 2299 seconds, 80.8% of all visitation time by insects. Out of all Apidae, *Bombus pensylvanicus* were present for 1314 seconds (57.2%) and *Melissodes desponsa* were present for 935 seconds (40.7%). *M. desponsa* and *B. pensylvanicus* constituted almost 98% of total Apidae visits.

Apical meristem mining did not influence the activities of bees in the Apidae on tall thistle flower heads. The length of visit for bees in the Apidae was not significantly different between the two sites (χ^2=0.84, df=1, P=0.35) nor did it differ among treatment levels (I vs. C: χ^2=0.043, df=1, P=0.83). Average time spent by a member of Apidae per plant was 70 seconds ±27.3. Also, there was no difference between the insecticide and control plants in the total time spent by bees in the Apidae (i.e. the sum of the time spent during all visits by Apidae individuals to a thistle plant) (χ^2=0.000, df=1, P=1) on the plant's flower heads or in total number of Apidae individuals (χ^2=0.036, df=1, P=0.54) that visited a plant's flower heads. Also, the probability that a plant would receive one or more visits by a member of Apidae did not differ between the control and insecticide plants (χ^2=0.12, df=1, P=0.73). Finally, total number of morphotaxa (all the insects including Apidae) seen visiting flowers per minute observed was not significantly different between the insecticide and control plants (I vs. C: χ^2=0.12, df=1, P=0.72).

From both my collections and timed floral visitor observations, it was apparent that bee activity was associated with weather conditions. During the visitation of bees, weather was normally warm or sunny, which is favorable for bees' activity. However, there was a very low (or no) frequency of thistle flower visits (or low activity of bees) during the cool early morning, rainy, cloudy and very windy weather. Also, bees were more active in the morning rather than during the very hot afternoon that are not favorable for the bees' activity.

3.5 Self-Compatibility

At least one flower head of 12 plants, out of 20 plants that began the experiment, was damaged or lost and not available for the analysis. So, only 8 pairs were analyzed. There was no ($t= -0.71$, $df=7$, $p=0.502$) difference between bagged and non- bagged flower heads in their diameter (Bagged=17.98 mm±0.79, un-bagged=17.26 mm ±0.98). The difference in total seeds (including shriveled and unfilled seeds) produced by bagged and by unbagged flower heads was significant (Fig. 18; $t=-2.48$, $df=7$, $p=0.042$) with more seeds produced in bagged flower heads. Total shriveled seeds (damaged and undamaged), which may represent aborted ovules, were significantly more abundant in bagged heads than in non-bagged heads (Fig. 19; $t= 4.28$, $df=7$, $p=0.0037$). However, the number of filled seeds per flower head, which are considered viable, was significantly less in bagged heads than in non-bagged heads (Fig. 20; $t= -3.03$, $df=7$, $p=0.019$). Eleven of 23 flower heads dissected were damaged internally by insects showing a strong potential for flower head-feeding insects to influence the number of viable seeds produced per flower head. There was no significant ($t=-1.43$, $df=7$, $p=0.197$) difference in damage of the filled seeds produced by bagged and unbagged heads.

CHAPTER 4

DISCUSSION

4.1 Does Apical Meristem Mining Affect Plant Fitness?

Apical meristem mining affected maternal fitness in *Cirsium altissimum*. Effects of apical meristem mining on *C. altissimum* plants' maternal fitness resulted from both reduced survival to flowering and reduced seed production by surviving plants, but not from reduced quality of the off-spring produced.

Apical meristem mining, despite the small amount of tissue lost from the plant, significantly increased the mortality rate of *C. altissimum* plants preventing many individuals from producing seeds in their lifetime. Insect herbivores have often been hypothesized to be less detrimental to plants than vertebrate herbivores because insects may consume a very small amount of plant tissue with compared to vertebrates (Crawley 1989, Hulme 1994). However, the type of plant tissue damaged can be important in determining herbivore effects. Large herbivores may just graze or damage the leaves but smaller herbivores can specifically target the meristem tissues (Ehrlen 1995). Being rapidly growing, soft tissues and being a more nutritious part of the plant, meristems are more prone to damage from wide range of herbivores and this damage has more significant effects on plants than any other herbivory (Ehrlen 1995, Wise and Abrahamson 2008). So, even a small tissue loss of this vital organ can have a profound effect on plant fitness (Wise and Abrahamson 2008) because it reduces the growth rate during the most favorable time and plants have to activate new meristem tissues instead of accumulating the resources for plant growth (Marquis 1992, Ehrlen 1995).

Relatively few studies have experimentally quantified the effects of apical meristem mining upon the mortality of host plants in the field. Apical meristem removal by deer herbivory (Brody et al. 2007) and by clipping (Brody and Irwin 2012) both caused higher mortality in *Ipomopsis aggregata* plants. Ehrlen (1995) found that among three different forms of herbivory (meristem damage by molluscs, grazing by vertebrates and folivory by insects) on *Lathyrus vernus*, meristem damage had the most severe effect on plant performance, reducing plant survival. In contrast, loss of other plant parts may not have such detrimental effects on survival (Ehrlen 1995, Andrieu et al. 2011).

Not only did apical meristem mining increase adult tall thistle mortality rates, but live plants with mined apical meristems that were able to set seeds produced significantly fewer seeds (by 30% in site A and 37% in site B) than plants that were not mined. My result is not surprising compared with previous studies. Shoot damage (simulated grazing) reduced seed production by 55% in *Pimpinella saxifraga* (Huhta et al. 2009). Similarly, along with increased mortality, a 50% reduction in total seed production due to clipping the apical meristem in *Ipomopsis aggregata* was reported by Brody and Irwin (2012).

After damage to the apical meristem, *C. altissimum* cannot compensate fully. In their review study, Wise and Abrahamson (2008) found that full compensation was prevalent only in high resource conditions. Very few (2/18 of the studies they reviewed) studies in plant-herbivore interactions showed full compensation under ambient or low nutrient conditions (Wise and Abrahamson 2008). The habitat of *C. altissimum* plants is not highly nutrient rich so it is unlikely that these plants can compensate for any huge loss on their tissues.

However, some studies have shown that plants that suffer apical meristem mining can compensate or overcompensate in seed production. Lortie and Aarssen (2000) found that,

because of more lateral branches, *Verbascum thapsus* plants with apical meristem damage produced significantly more seeds than undamaged (un-branched) plants possibly showing over-compensation. Earlier studies with *V. thapsus* showed that despite the morphological and phenological effects of apical meristem damage there was no effect on seed production (Naber and Aarssen 1998). Nevertheless, neither study involved sowing seeds to evaluate whether those seeds were viable or not nor did any study record the off-spring vigor.

Higher production of viable seeds in plants can be crucial to determining plant population dynamics as seedling recruitment can be determined by the number of viable seeds (Louda 1982). Late developing seed by late flowering plants may not get enough time to mature (Benner 1988), which can heavily decrease the number of viable seeds. For short lived, perennial, monocarpic plants that have a transient seed bank, like *C. altissimum,* any form of insect herbivory that limits seed production may be a critical limiting factor for plant population growth (Louda 1994, Maron and Crone 2006). Russell et al. (2010) found that in 2007, addition of viable seeds increased the numbers of seedlings and reproductive adults of *C. altissimum* in the next generation at 5 sites in southeast Nebraska restored tallgrass prairie suggesting that this monocarpic perennial plant is seed limited.

Despite the greater number of seeds produced by undamaged *C. altissimum* plants, germination rate of seeds and average seedling biomass did not differ significantly between damaged and undamaged plants. This outcome may occur because only the filled seeds, which are supposed to be viable, were selected to sow in the greenhouse. Also, it may be because of the unequal sample sizes. There were very few control plants fallen under the criteria of sowing seeds. An average germination rate of >86% across all flower heads shows a high degree of seed viability in *C. altissimum*, irrespective of their herbivory history during their previous generation.

Also, out of 30 flower heads with 100% germination rate, 66% (20/30) were the 100 heads (which constituted only 28.3% of the total flower heads) showing the seeds produced by apical heads or early flower heads were more viable than the seeds produced by late flower heads.

Examining seed germination rates and seedling biomass, as an indicator of off-spring vigor, is a step closer to assessing off-spring quality than is evaluated in most herbivory studies. However, unless off-spring survive to produce their own seeds, increases in numbers of seeds produced by the parents or the higher seed germination rates may not translate into higher fitness of the parents. Only one previous study was found that quantified germination rates of seeds produced by plants with damaged vs. undamaged apical meristems (Naber and Aarssen 1998). Naber and Aarssen (1998) did not find any effect on germination rates of seeds produced by clipped (apical meristem damaged) *Verbascum thapsus* plants, which is consistent with my results. However, no study has been found reporting the seedling vigor produced by damaged and undamaged plants. Even though, seed germination rate and seedling vigor were not significantly different between damaged (mined) and undamaged plants (unmined), it seems very likely that apical meristem mining affects *C. altissimum* fitness since the damaged plants had significantly higher mortality rates and lower seed production.

4.2 Community Composition of Floral-Visiting Bees on *Cirsium altissimum*

Based on the very high percentage of bees (especially Apidae) in collections that I made from *C. altissimum* flowers (Table 2), it is very likely that major pollinators of this plant are bees, particularly *Melissodes desponsa* and *Bombus pensylvanicus*, which comprise >72% of Apidae specimens collected. Also, these two species were abundant almost throughout the tall thistle flowering season and throughout the day, thereby synchronizing with the whole flowering

period of *C. altissimum*. Other bees were relatively less abundant and their activities were highly specific with the time of day as well as flowering season (Table 2). In my floral visitor observations most of the time *M. desponsa* and *B. pensylvanicus* were collecting pollen voraciously from *C. altissimum* plants. They were observed carrying abundant pollen in their pollen baskets which may indicate their very important role of pollinating the *C. altissimum* flowers. However, no studies were done on the pollen composition to prove it.

There are very few studies in *Cirsium* species regarding their flower visitors. Powell et al. (2011) found that 6 *Cirsium* species (1 invasive and 5 native) were primarily visited by *Bombus* with few *Apis melifera* and other solitary bees. However, the species of *Bombus* (*B. vosnesenskii*) they found was not present in my site nor did these authors find any *Bombus* species that I found on *C. altissimum*. This lack of overlap in bee species may be because of the different geographical locations, climates and the habitat types in California where they studied and Kansas where my research was conducted. Nevertheless, it seems that *Bombus* is one of the major pollinators of *Cirsium* species in U.S.

Bombus pensylvanicus has been categorized as 'uncommon' and 'possibly in decline' from the eastern U.S. (Colla et al. 2011) and *B. pensylvanicus* subspecies *sonorous* is 'uncommon' in the western U.S. (Koch et al. 2012). In contrast, *B. pensylvanicus* was the second most commonly collected bee from *C. altissimum* in my study site and was >3X more frequently collected than all other *Bombus* species combined. Further, no papers that I found mentioned *Cirsium* species as a selected food plant of *B. pensylvanicus* despite the fact that it is very commonly found on *C. altissimum* at my study site. Still further, this species is commonly found on *Cirsium* species in Arkansas and Tennessee (Amber Tripodi, *Pers. Comm.*). It is not surprising to see *Melissodes desponsa* as the most commonly collected specimen from *C.*

altissimum flowers since it is believed to be one of the most common visitors of *Cirsium* species (Dr. Charles Michener, *Pers Comm*).

4.3 Are effects of Apical Meristem Mining on Maternal Fitness Mediated by Changes in Floral Visitation?

Because I found that tall thistle is largely self-incompatible, changes in floral visitation that affect pollen transfer among plants could strongly affect seed production. In documented examples of herbivory altering floral visitation to the shared host plant, these plant-mediated effects of herbivores on floral visitation often arise through herbivory-induced changes in plant architecture and phenology (Gronemeyer et al. 1997, Kliber & Eckert 2004, Brody and Irwin 2012). Further, damage to apical meristems often has been associated with large changes in plant architecture, most often with reduced plant height and increased numbers of branches and flower heads per plant, and changes in flowering phenology. In *C. altissimum*, however, changes in plant architecture and, especially, phenology had no effect on patterns of floral visitation by the largest group of floral visitors, bees in the Apidae.

In agreement with my results, previous studies of apical meristem mining often have found reduced plant height because of the apical meristem damage. Hamback et al. (2011) found that damaged *Scrophularia nodosa* plants were shorter than undamaged plants. Reduction of *C. altissimum* plant height may be because the damaged plants lost resources and it took time to re-grow. So, slower growth rate after the plants suffered damage may cause the shorter heights (Brody et al. 2007). Since the *C. altissimum* plants were grown in low resources conditions, they may never be able to compensate fully. More importantly, an apical head on the main shoot is vertically oriented but the lateral branches are shorter and slanted at $60\text{-}70^0$ instead of having a

90^0 orientation. Therefore, the absence of an apical flower head can, by itself, account for some reduction in plant height. Tall plants with high apical flower heads, which is more prevalent in undamaged *C. altissimum* plants, would be beneficial for plants getting more access to light and being more visible to pollinators (Naber and Aarssen 1998, West 2012).

Number of flower heads and branches per plant, another aspect of plant architecture, was not changed by apical meristem mining in *C. altissimum*. Therefore, reduced seed production by mined plants cannot be attributed to fewer inflorescences produced. In contrast, many previous studies of apical meristem mining report increases in branches and so the flowers on these branches after the plants were damaged (Benner 1988, Naber and Aarssen 1998, Lortie and Aarssen 2000, Wise and Abrahamson 2008). However, in *Ipomopsis aggregata* despite the increase in branches, because of apical meristem damage by deer herbivory, there were fewer flowers produced (Brody et al. 2007). Interestingly, apical meristem mining of tall thistle plants increased the proportion of axial heads as compared to terminal flower heads by 254.55 %. Since apical meristem miners damaged the apical meristem or tip of a main stalk or tip of any other branches, it may be the reason why there were more axial heads in damaged plants. The lack of difference in number of branches on *C. altissimum* between mined and unmined plants may occur because naturally all *C. altissimum* plants develop branches irrespective of the herbivory. When there are more branches, there are definitely more flower heads on them. On the other hand, even if apical meristem has released in the damaged plants, they may not have enough sources or capacity to develop more branches (and so more flower heads) than when the apical meristem was not damaged. However, since there was no differences in number of branches between mined and unmined plants but the heights of mined plants were shorter, there was a trend of producing higher branch density in damaged plants.

Perhaps more importantly than the total number of flower heads produced per plant, the number of flower heads that were bloomed was marginally significantly higher (22 % more in site A and 30% more in site B) in undamaged plants than in the damaged plants despite the fact that there was no significant difference in the total flower heads (Table 1). This may show a trend which is possibly a reason why more seeds were produced in undamaged plants. For plant fitness, number of flower heads that bloom would be more valuable than just the total number of flower heads produced (bloomed and un-bloomed both).

Floral display size (number of flower heads bloomed simultaneously) was also significantly reduced by apical meristem mining in *C. altissimum*. It may be because the damaged plants had fewer resources to mature flower heads that were initiated or because damaged plants had less time to bloom their flower heads because of the reduced flowering duration. Ohashi and Yahara (1998) found that per head visitation rate by bumble bees, which are essential for the pollination/seed success of *Cirsium purpuratum*, was independent with the display size. Even though there are many cases where flower visitation is independent of the size of the floral display (Ohashi and Yahara 1998, Grindeland et al. 2005), flower visitation was affected, most often positively, by floral display size in many studies on bee pollinated plants (Robertson and Macnair 1995 in *Myosotis colensoi* and *Mimulus guttatus*, Ohashi and Yahara 1998 and 2002 in *C. purpuratum,* Krupnick and Weis 1999 in *Isomeris arborea*, and Grindeland et al. 2005 in *Digitalis purpurea*).

Flowering phenology of *C. altissimum* was very strongly affected by apical meristem mining. Changes in flowering phenology can have strong negative effects on plant reproduction (English-Loeb and Karban 1992). In entomophilous flowering plants, like *C. altissimum*, availability of pollinators during the flowering season is crucial for pollination. A shortened

flowering period or delayed flowering may result in less chance of getting pollinated as pollinators have their own seasonal phenology that may no longer synchronize with the host plant's flowering. Some undamaged *C. altissimum* plants bloomed for up to 10 weeks, which might increase the plant's chances to get visited by pollinators as compared to damaged plants whose flowering duration never exceeded 5 weeks. This may ultimately negatively affect seed production and plant population dynamics.

Changes in dates when plants initiate flowering often have been observed with apical meristem damage. Banta et al. (2010) found that fruit ripening and flowering was delayed because of apical meristem damage by clipping in *Arabidopsis thaliana*. Similarly, because of the damage to the apical meristem by clipping or by deer browsing, *Campanulastrum americanum* had delayed flowering with negative effect in plant fitness (Lin and Galloway 2010). Perennial plants can compensate for tissue or seed production lost to herbivory immediately under high resources conditions but may postpone their flowering or reproduction for the next year under low nutrient condition (Huhta et al. 2009). However, monocarpic plants like *C. altissimum* may not be able to postpone their reproduction until next year once they attempt reproduction by producing a flowering stalk. So, the only option remaining to these plants is having a reduced flowering period because of the delayed flowering.

Several previous studies have shown that changed flowering phenology, caused by apical meristem mining, can affect plant-pollinator interactions, plant-seed predator interactions and plant performance as changed flowering phenology may disrupt the synchrony of plant-pollinator interactions (Kliber & Eckert 2004, Rafferty and Ives 2011). In a meta-analysis, Elzinga et al. (2007) found that most pollinators favor early flowering and peak flowering patches. Apical meristem damage (by clipping or by deer browsing) in *Campanulastrum*

americanum delayed flowering which caused a decrease in offspring in the next generation with a negative effect on plant fitness (Galloway and Burgess 2009, Lin and Galloway 2010). After the apical meristem was damaged by mule deer (*Odocoileus hemionus*) browsing and by clipping, *Ipomopsis aggregata* had delayed flowering that causes less seed production because of less overlap with pollinators' peak activity (Freeman et al. 2003, Brody and Irwin 2012). However, unlike many of the previous studies, the reason for the non-significant effect of changed flowering phenology in tall thistle upon the pollinator's activity may be because the major pollinators of *C. altissimum* were available to pollinate the plants throughout their flowering season.

Flower visitor observations showed that there was no influence of apical meristem mining on the behavior of Apidae that visited *C. altissimum* flower heads. This study is consistent with several previous studies that have shown effects of insect herbivory on plant architecture and/or flowering phenology but no effect on pollinator's activity (Ohashi and Yahara 1998, Grindeland et al. 2005, Brody and Irwin 2012). There are both biological and methodological reasons why herbivore-induced changes in architecture and phenology may not have translated into effects on floral visitation patterns in *C. altissimum*. Biologically, since the prime pollinators (*Melissodes desponsa* and *Bombus pensylvanicus*) were available in the field throughout the flowering season (Table 2) of *C. altissimum*, there was no effect on synchrony despite delayed flowering in damaged plants. Changes in plant architecture, specifically plant height, might not have translated into effects on floral visitation because in a drought year perhaps the vegetation surrounding the tall thistle plants was shorter, so even relatively short tall thistle plants readily stood out amid drought-stressed, short grasses. The presence of relatively short surrounding vegetation, because of drought, might make reductions in plant height due to

apical meristem mining less important in reducing visibility of the plant. Methodologically, the lack of a relationship between apical meristem mining and floral visitation may be because the sample size for floral visitor observations was too small (11 hours only) to detect relatively subtle changes in visitation.

The changes in architecture and flowering phenology of *C. altissimum* adults did not affect floral visitor behavior and, hence, likely did not drive indirect effects of apical meristem mining on seed production. However, these changes in host plant architecture and phenology may provide mechanisms underlying direct effects of the apical meristem mining on seed production. Also, these changes in *C. altissimum* may be interrelated and may have interacting effects on plant performance. The shift in flower head production on *C. altissimum* with mined apical meristems from terminal heads to axial heads may reduce seed production if axial heads are smaller and have less potential seed production. No study was found comparing the number of seeds produced by axial heads vs. terminal heads. Lower seed production by axial heads may occur because resources are less available to these axial heads, which are not directly attached or not closely situated near the resource producing plant parts like leaves, stem etc. Further, these axial flower heads are produced after terminal heads on the same branch when plants may not have sufficient resources to allocate for the axial heads. In resource limited conditions plants' regrowth or attempt to compensate may not be sufficient (Huhta et al. 2009), so it takes more time for re-growth and compensation for tissue loss when the plants are damaged (Freeman et al.2003, Brody et al. 2007). Also, since there may be competition for the limited resources among flower heads; early flower heads may preempt resources (Kliber and Eckert 2004). Early flower heads in tall thistle may have both spatial and temporal benefits over late flower heads (Diggle 1997, Kliber and Eckert 2004).

Production of late flower heads (lateral or axial) is strongly associated with the flowering phenology of plants. *C. altissimum* has determinate flowering in which apical flower heads are always the early flowers and the lateral and then axial flower heads (as described above) are the late and even later flowers respectively. Lin and Galloway (2010) also found that lateral branches (which otherwise may not be produced) and so the lateral flower heads on them, were developed only after the apical meristem was damaged in *Campanulastrum americanum*. Effects of apical meristem mining in delaying flower head production in *C. altissimum* also may provide a mechanism of direct effects, not mediated by floral visitors, on tall thistle seed production. Tall thistle's late flowering phenology (August-September) as compared to other thistles might preclude compensatory flowering. Perhaps a plant species that flowers earlier in the growing season, as discussed above, would be more likely to fully compensate for apical meristem mining because there would be more time to produce compensatory flower heads.

4.4 Future Research Directions

From the previous discussion, apical meristem mining seems very effective in reducing plant fitness through increasing plant mortality rates and decreasing seed production of surviving plants. Interestingly, apical meristem mining had fewer changes in many of the morphological characters (i.e. total number of flower heads, branches and leaves) but there were strong effects on flowering phenology. Paradoxically, changes in plant architecture and flowering phenology did not affect the flower visitation but still seed production was affected despite the fact that *C. altissimum* is self-incompatible. So, to resolve some of these controversial results, it would be very worthwhile to do further studies.

It would be interesting to determine where and when apical meristem damage is likely to reduce host plant fitness and to identify the conditions under which herbivory is likely to alter floral visitation. Greater sample sizes with experiments conducted in more sites with an emphasis on interactions of apical meristem mining with damage by other guilds of herbivores like folivory, florivory and mammalian herbivory would give more strength to these results. I think that damage by other herbivore guilds can influence the extent to which *C. altissimum* is capable of compensating for apical meristem mining because different herbivory may have some interacting and additive effects on plant performance (Irwin and Brody 2011). Besides interactions with other forms of herbivory, abiotic factors like soil nutrient availability, precipitation, temperature may have been interacting with the apical meristem mining and other herbivory to influence their effect on plant performance. Strauss et al. (2001) also found that environmental factors (soil quality etc) affected plant fitness in *Raphanus raphanistrum*. Plants growing/flowering in a drought year may respond differently than plants grown in a normal year. For example, growth rates, phenology and herbivory stress can be different under favorable conditions that can also have different effects on pollinators' activity even though I did not see during 2012, a dry year. Many studies have shown that plants can compensate (or even overcompensate) easily if they are growing under high resource condition but there are very rare cases of compensation under low nutrient conditions (Wise and Abrahamson 2008, Huhta et al. 2009). So, working simultaneously with different forms of herbivory on *C. altissimum* in different climatic years and in different soil productivity in multiyear studies might give a more general result than from a one or two year studies.

To more accurately assess effects of apical meristem mining on plant maternal fitness, it would be better to quantify the long-term performance of offspring produced by mined and un-

mined plants. For example, Agrawal (2001) demonstrated trans-generational effects of herbivory in that offspring of damaged *Raphanus raphanistrum* were more susceptible with pathogens. I germinated *Cirsium altissimum* seeds successfully in the greenhouse but did not have time to determine the rate at which seedlings that germinate from damaged and undamaged plants ultimately produce seeds. I would also be interested with the same kind of study (that I did in 180 experimental plants) again on their offspring until they produce the seeds to see whether there is any effect of herbivory history in the next generation. Thus, the multiyear and multigenerational study would give a clearer trend in plant population dynamics (Ehrlen 1995).

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APPENDIX

APPENDIX

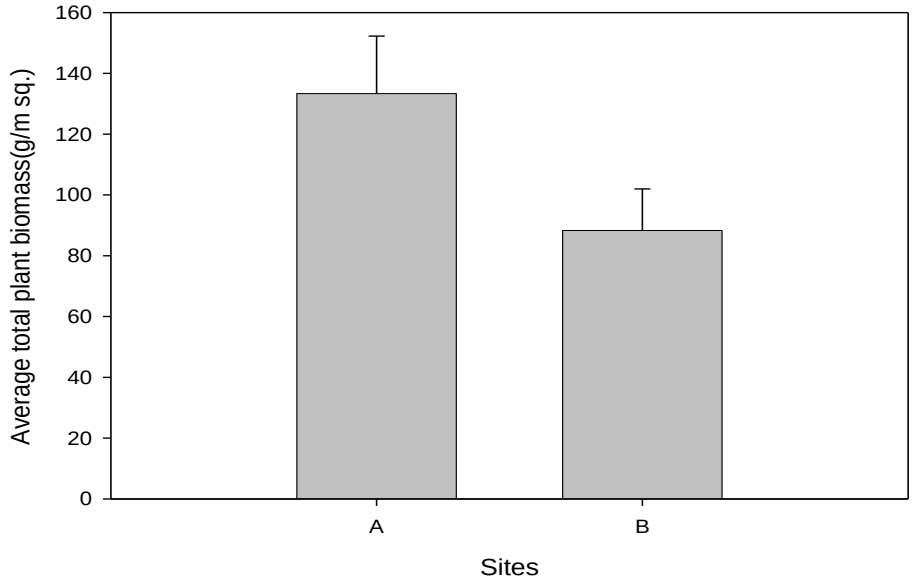

Figure 2. Mean (± standard error) total aboveground plant biomass at the two study sites at the Ninnescah Reserve. Plant biomass was collected in late September 2012.

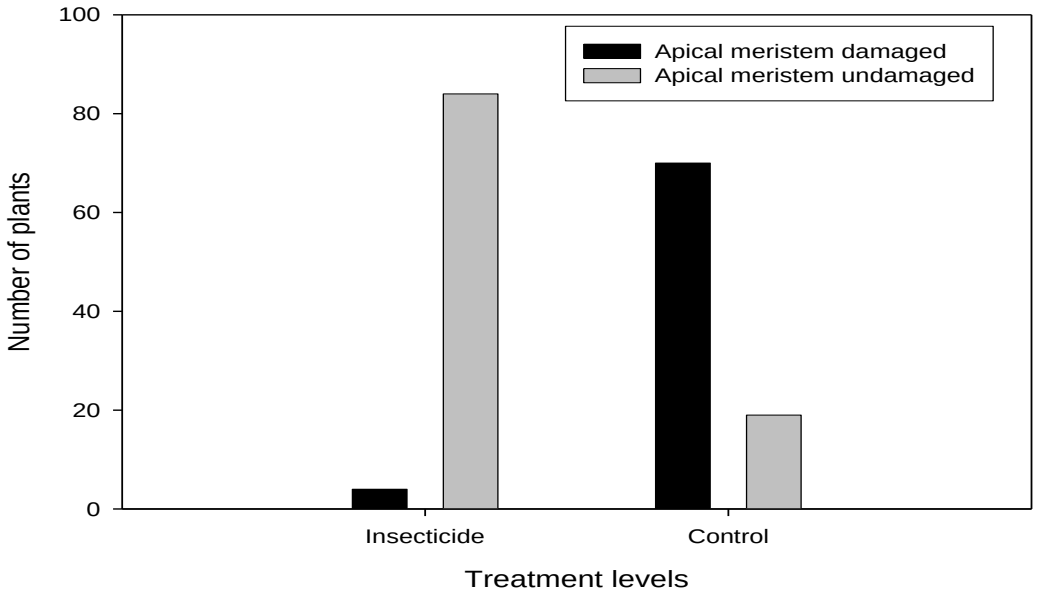

Figure 3. Number of experimental *C. altissimum* plants that had their apical meristem damaged by insects before producing an apical flower head. Plants were pooled across sites and across water and unmanipulated controls.

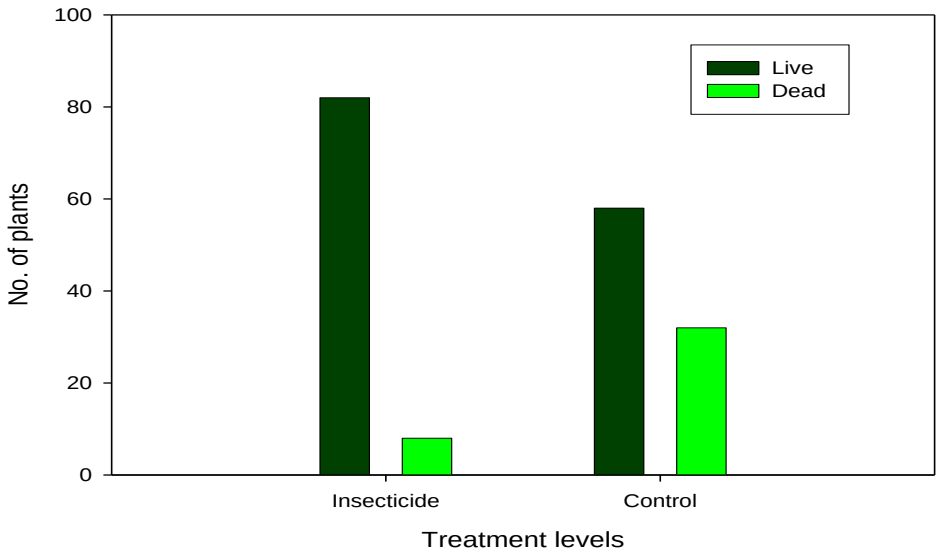

Figure 4. Number of *C. altissimum* plants that lived to disperse seeds vs. number of plants that died before dispersing seeds for insecticide treatment and for control plants. Plants were pooled across sites and across water and unmanipulated controls.

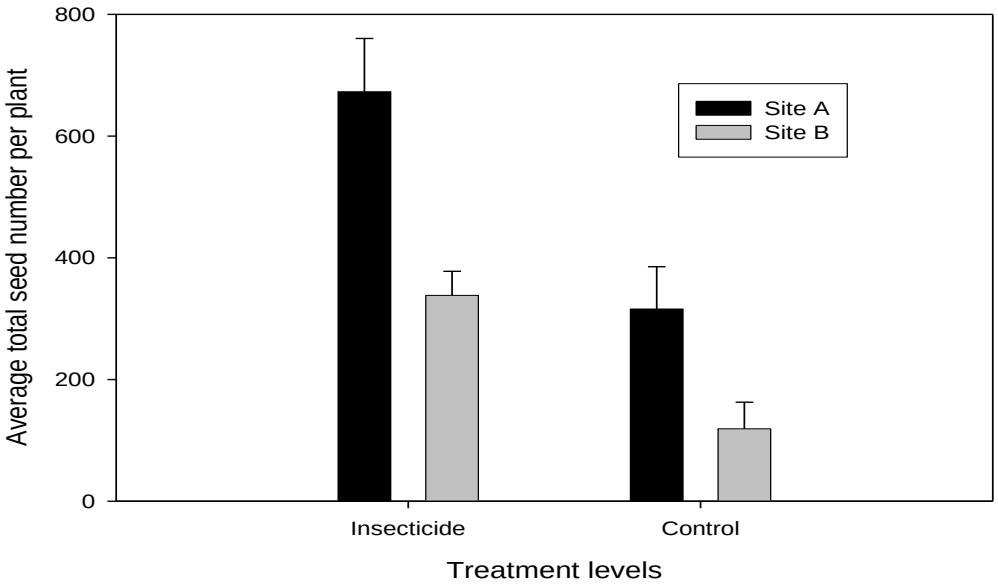

Figure 5. Mean (±standard error) number of undamaged, viable seeds produced by experimental *C. altissimum* plants. Means include plants that died before reproducing. Plants were pooled across water and unmanipulated controls.

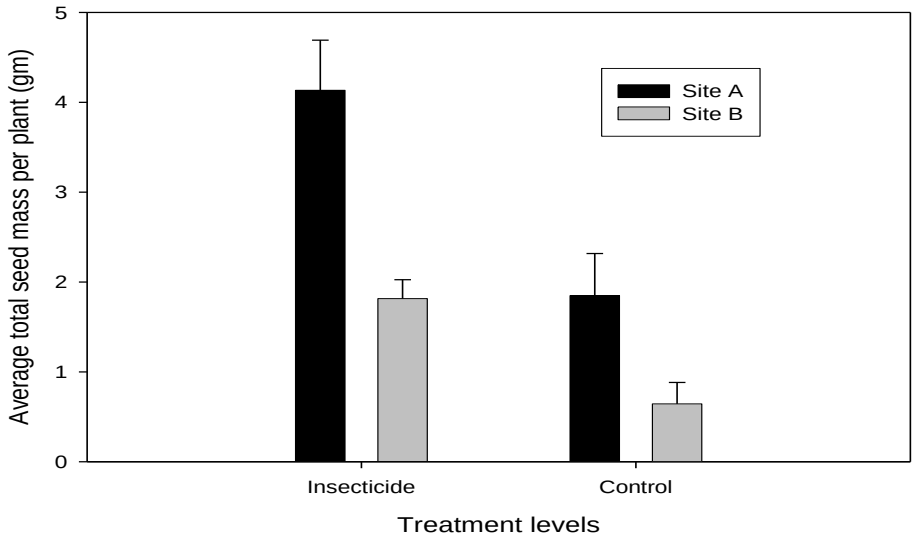

Figure 6. Mean (±standard error) total seed mass (weight of all undamaged, viable seeds of all flower heads of each plant) produced by insecticide treated and control *C. altissiumum* plants. Plants that died before reproducing are included. Plants were pooled across water- and unmanipulated controls.

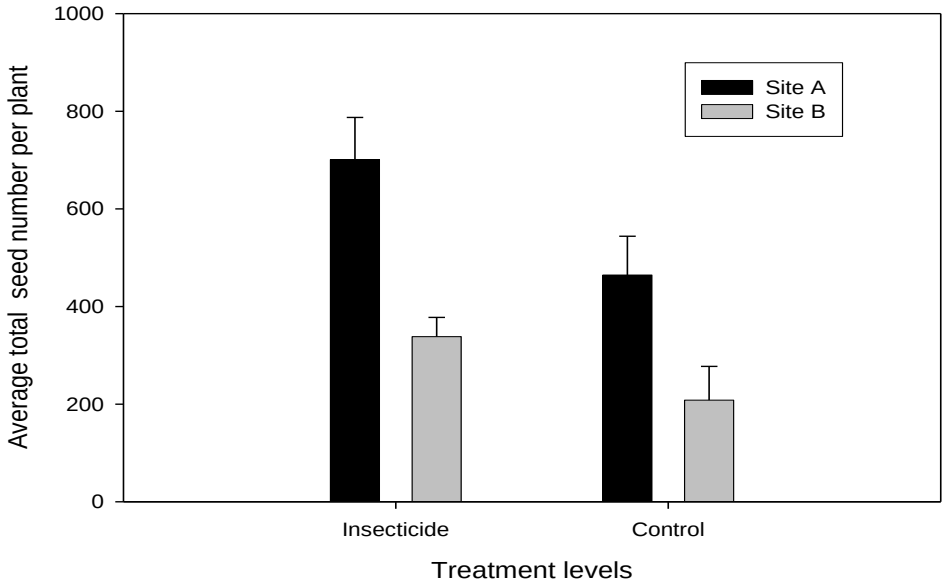

Figure 7. Mean (±standard error) total number of undamaged, viable seeds produced by insecticide-treated and control experimental *C. altissiumum* plants that survived to flower. Plants that died before flowering are not included. Plants were pooled across water- and unmanipulated controls.

Figure 8. Mean (±standard error) total seed mass (weight of seeds of all flower heads of each plant) produced by insecticide-treated and control experimental *C. altissiumum* plants that survived to flower. Plants that died before flowering are not included. Plants were pooled across water- and unmanipulated controls.

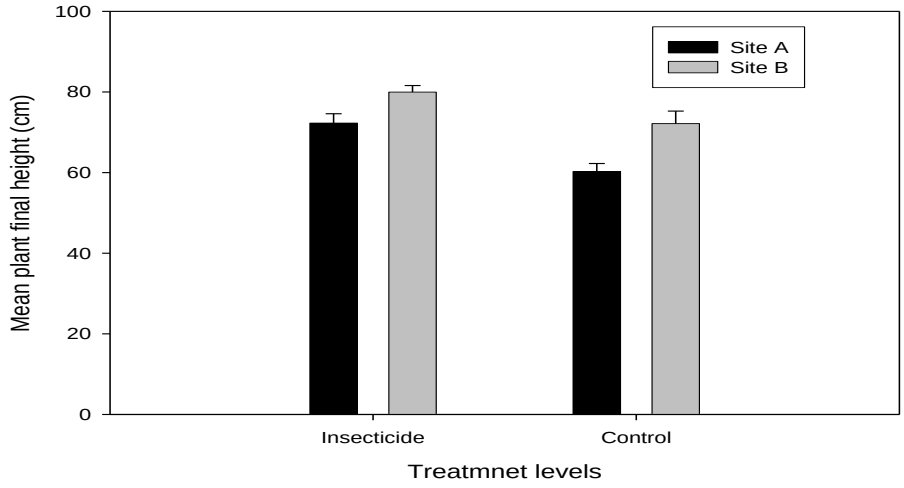

Figure 9. Mean (±standard error) plant height (cm) of insecticide and control *C. altissiumum* plants. Plants were pooled across water- and unmanipulated controls.

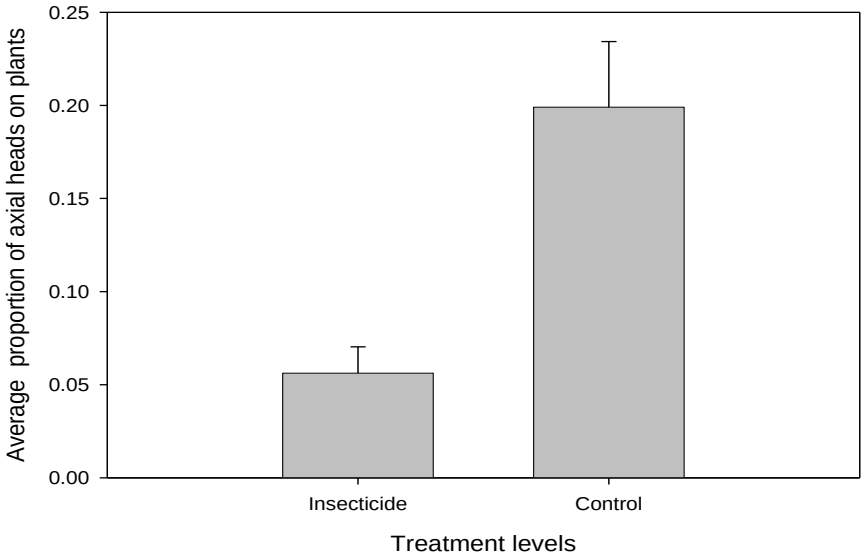

Figure 10. Mean (±standard error) proportion of axial heads among the total heads bloomed in insecticide and control *C. altissiumum* plants. Axial flower heads are all heads that are not at the apex of a lateral branch. Plants were pooled across sites and across water- and unmanipulated controls.

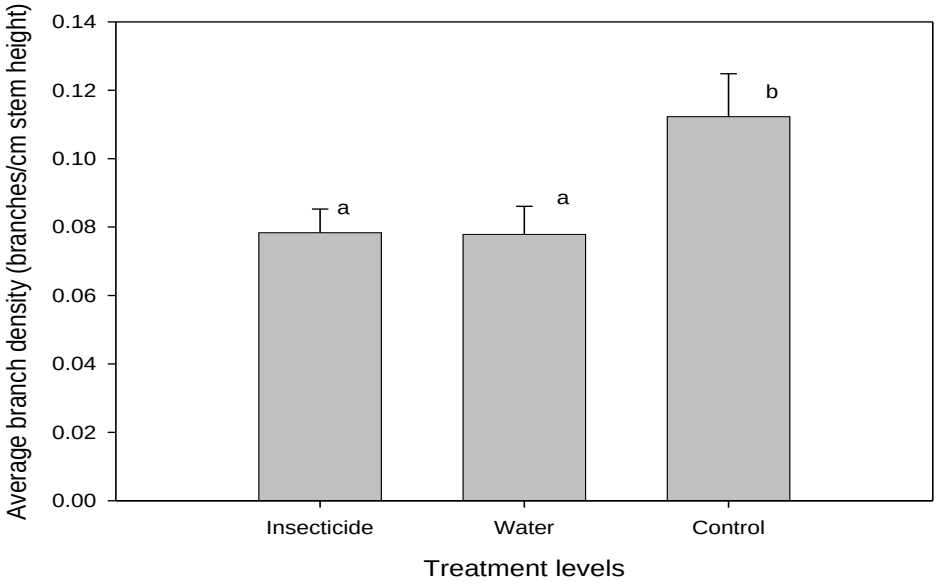

Figure 11. Mean (±standard error) branch density (total number of branches per plant height of experimental *C. altissiumum* plants in site A.

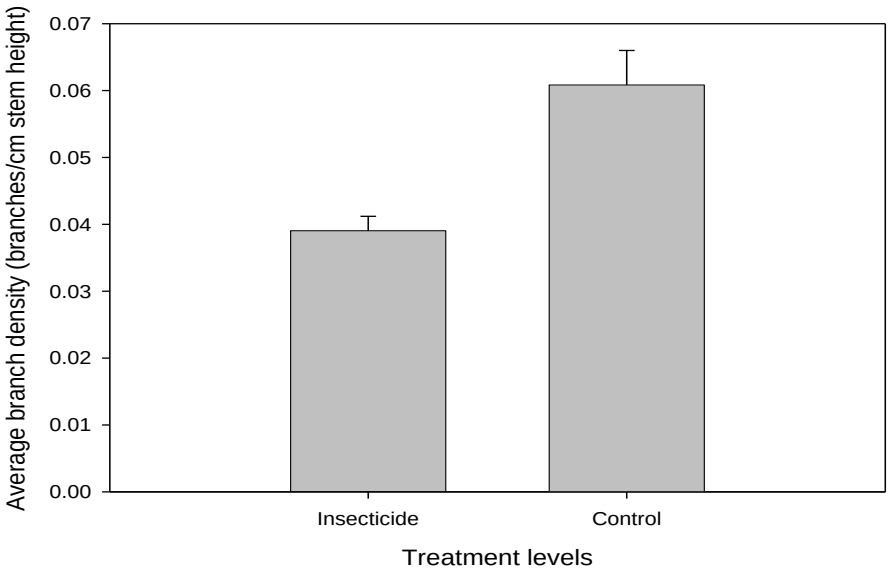

Figure 12. Mean (±standard error) branch density (total number of branches per plant height) of experimental *C. altissiumum* plants in site B. Plants were pooled across water- and unmanipulated controls.

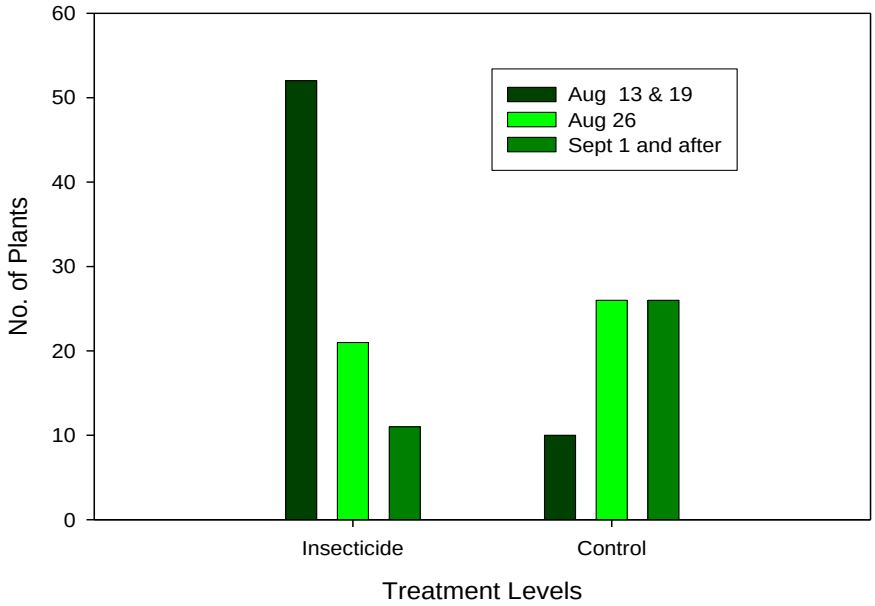

Figure 13. Initial dates of flowering for insecticide plants and control *C. altissiumum* plants. Plants were pooled across sites and across water- and unmanipulated controls.

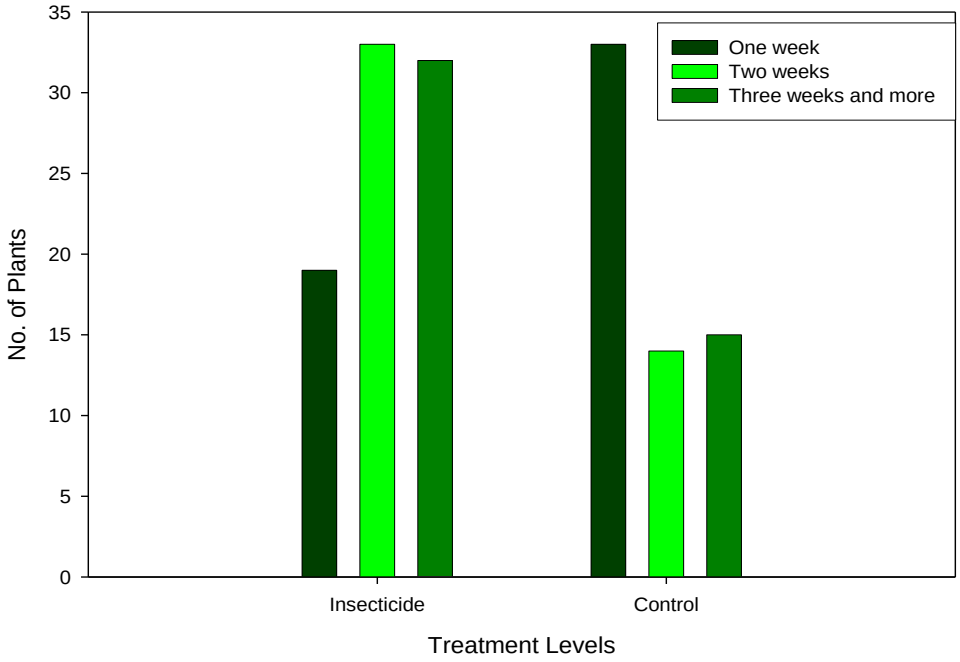

Figure 14. Duration of flowering for insecticide and control *C. altissiumum* plants. Plants were pooled across sites and across water- and unmanipulated controls.

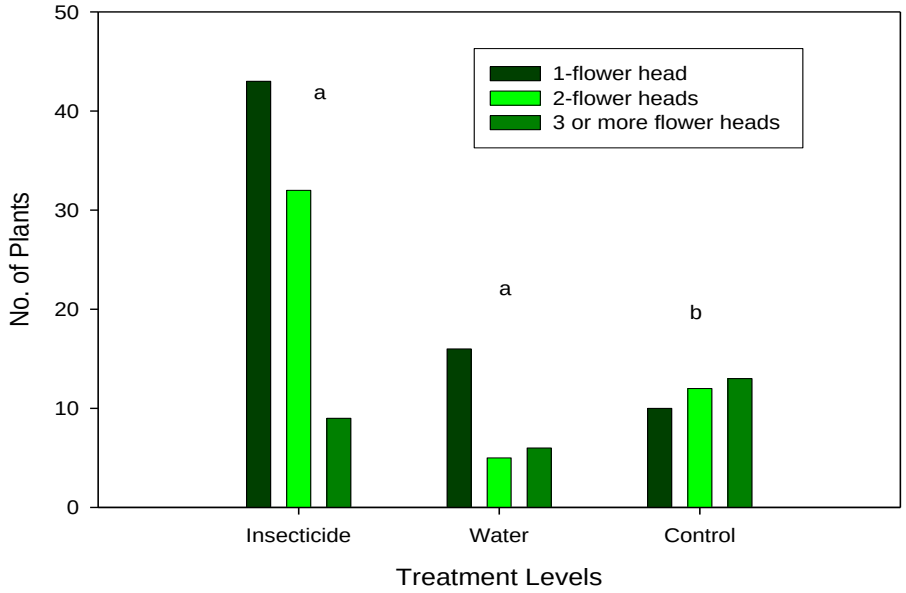

Figure 15. Maximum number of flower heads bloomed in a day for insecticide and control *C. altissimum* plants. Plants were pooled across sites and across water- and unmanipulated controls.

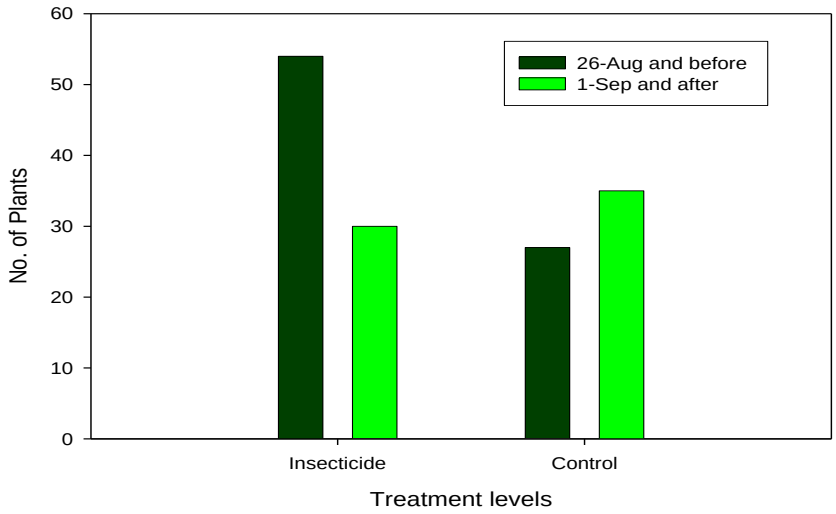

Figure 16. Dates when the maximum numbers of flower heads per plant bloomed in a day for insecticide and control *C. altissimum* plants. Plants were pooled across sites and across water- and unmanipulated controls.

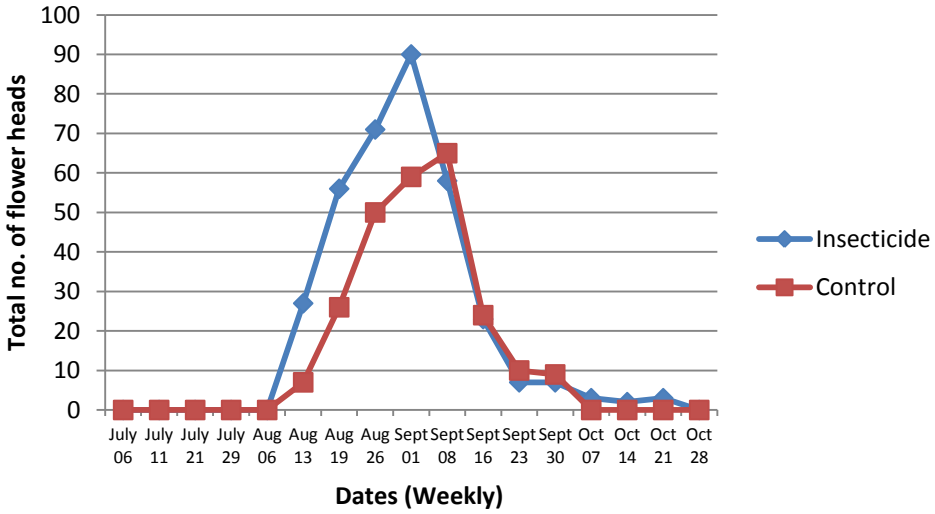

Figure 17. Floral displays of insecticide and control plants recorded in different dates throughout the flowering season of *C. altissimum*. Plants were pooled across sites and across water- and unmanipulated controls.

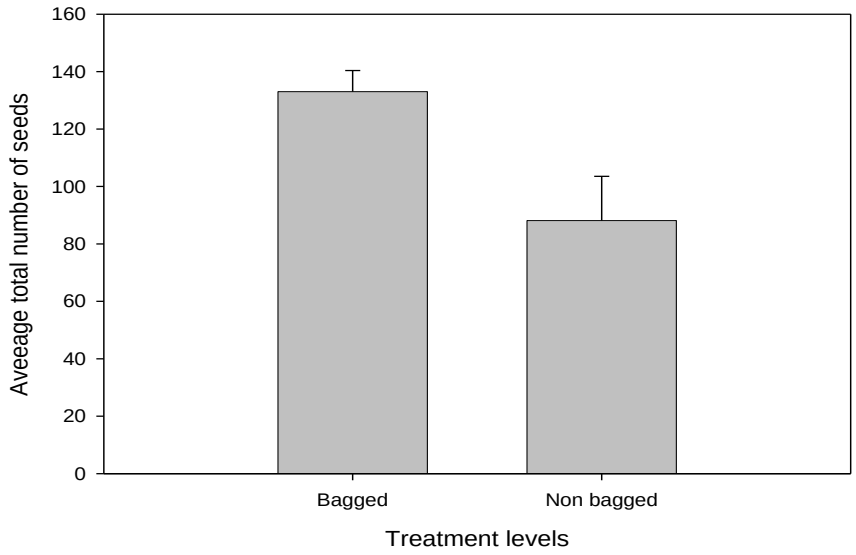

Figure 18. Mean (±standard error) total number of seeds produced by the bagged (pollinator exclusion) and non-bagged (pollinator access) flower heads in *C. altissiumum* in September 2011.

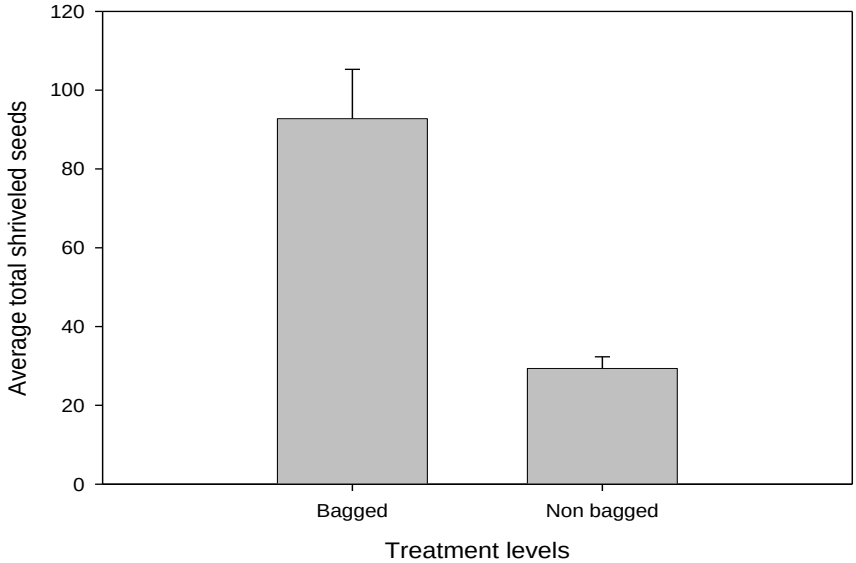

Figure 19. Mean (±standard error) total number of shriveled (aborted) seeds produced by the bagged (pollinator exclusion) and non-bagged (pollinator access) flower heads in *C. altissiumum*

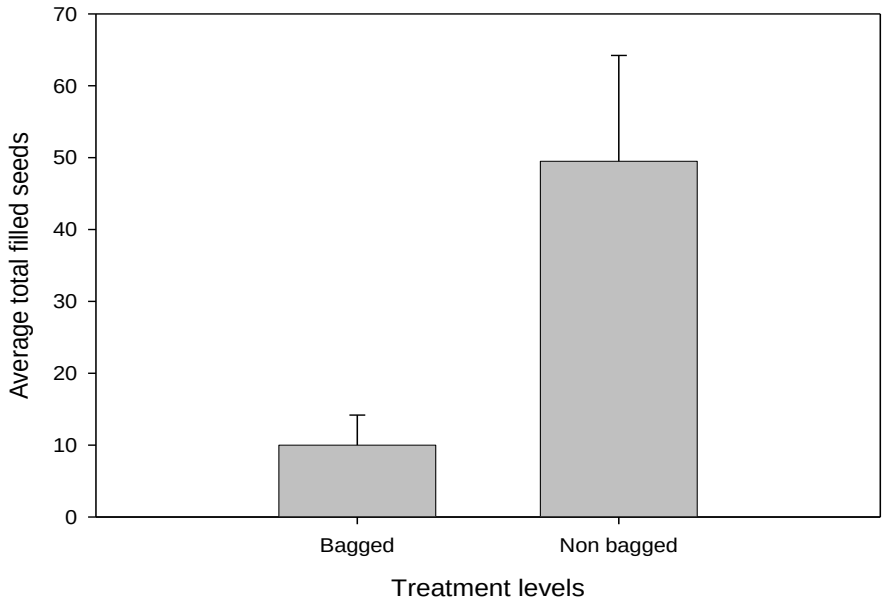

Figure 20. Mean (±standard error) total number of filled (viable) seeds produced by the bagged (pollinator exclusion) and non-bagged (pollinator access) flower heads in *C. altissiumum*.

Table 1. Summary of outcomes of statistical tests of effects of apical meristem mining on *C. altissimum* morphology. P-values with asterisk (*) are statistically significant ($p \geq 0.05$ for sites combined and $p \geq 0.025$ for sites not combined after Bonferroni correction).

Dependent variables		**Independent variables**		**Mean ± SE**	χ^2	**df**	**p-value**
Plant architecture	Total flower heads	I vs. W		I=8.51 ±0.70 W=9.41 ±2.04	0.89	1	0.34
	Final height (cm)	I vs. C	A	I= I=72.8 ±2.12 C=60.01 ±1.69	12.05	1	0.0005*
			B	I=80.30 ±1.64 C=71.85 ±2.31	6.45	1	0.011*
	Branches	I vs. W		I=4.52 ±0.36, W=4.30 ±0.41	0.045	1	0.83
	Leaves	I vs. C		I=37.8±1.49, C=48.06±4.58	0.399	1	0.52
	Total flower heads bloomed	I vs. W	A	I=5.35±0.58, W=4.18±0.99	3.7	1	0.05
			B	I=2.84±0.19, W=2±0.26	4.56	1	0.03
	Bloomed flower head density (bloomed heads per branch)	I vs. C		I=1.82±0.06, C=1.84±0.12	3.7	1	0.05*
	Branch density (branch per cm plant height)	I vs. W	A	I=0.078±0.007, W=0.078±0.008	0.03	1	0.85
		I vs. C	B	I=0.04±0.002, C=0.06±0.005	14.16	1	0.0002*.
	Proportion of axial heads	I vs. C		I=0.056±0.014, C=0.2±0.035	6.63	1	0.01*

Table 2. Species list of bees collected from *C. altissimum* flower heads. Presence of individual bee species on tall thistle flower heads in different weeks and times of day during the flowering season. N (both horizontally and vertically) is the number of specimens collected or represented and shaded boxes indicate the presence of species.

		Presence across flowering season							Presence at different times of day			
		August			September							
Species		2nd	3rd	4th	1st	2nd	3rd	4th	Morning (until 11am)	Around Noon (11-1pm)	Afternoon (1-5pm)	Evening (after 5 pm)
Site-A	**N**	**0**	**4**	**3**	**0**	**8**	**10**	**7**				
Anthophora walshii Cresson	2						■	■		■	■	
Bombus fervidus (Fabricius, 1798)	3							■		■	■	
Bombus pensylvanicus (De Geer,1773)	7		■				■	■	■	■	■	
Melissodes desponsa Smith	20		■	■		■	■		■	■	■	■
Site-B	**N**	**4**	**14**	**21**	**12**	**12**	**17**	**16**				
Agapostemon sp.	1											
Anthophora walshii Cresson	8					■	■		■	■	■	
Apis mellifera Linnaeus	3	■	■					■	■	■		
Bombus fervidus (Fabricius, 1798)	6						■	■	■	■	■	
Bombus fraternus (Smith, 1854)	2		■						■	■		
Bombus griseocollis (DeGeer, 1773)	1							■		■		
Bombus pensylvanicus (De Geer,1773)	31	■	■	■	■	■	■	■	■	■	■	■
Halictus ligatus Say	3		■	■					■	■		
Megachile sp.	3		■	■	■				■	■		■
Melissodes desponsa Smith	35	■	■	■	■	■	■	■	■	■	■	■
Triepeolus sp.	3		■		■				■			■