# The effects of defoliation on growth, reproduction and pollination of a non-native legume (*Astragalus cicer)* in the mixedgrass prairie

by

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# Abstract

Herbivory can lead to trade-offs between plant growth and reproduction as defoliated plants lose initial resource investments and have reduced photosynthetic capacity. This trade-off can lead to reduced investment into reproductive structures and have repercussions for floral traits, pollinator attraction and plant reproduction. I investigated the effect of timing and intensity of artificial herbivory on the growth, floral traits, pollinator visitation and reproduction of an exotic perennial legume, *Astragalus cicer L*. My study was conducted in a native mixedgrass prairie grazed by cattle. Over two summers, I clipped plants and measured the production of inflorescences, pollinator visitation, and production of vegetative and fruit biomass to evaluate the impact of low or high intensity clipping either earlier or later in the growing season. In 2015, I additionally measured nectar production to better understand the effect of clipping on floral components.

In my first study, I found that high intensity clipping led to smaller plant spread, but leaf biomass did not differ among treatments, and in 2014, high intensity clipping produced more stem growth compared to low intensity treatment. Late-high intensity clipping also produced more vegetative biomass in 2015 compared to 2014. In 2014, late-high intensity clipping treatments led to lower fruit biomass and late clipping treatments led to a lower fruit to vegetative biomass ratio. My results demonstrate that *Astragalus cicer L.* is able to withstand a higher clipping intensity with limited impacts on vegetative growth. Further, when plants are clipped earlier, prior to flowering, there is minimal impact to reproduction.

In my second study, I found that late-high intensity clipping led to fewer numbers of inflorescences, while a late clipping intensity negatively affected nectar sugar per flower, fruit and seed production. However, treatments had a minimal effect on pollinator visitation. Aside from effects on nectar production, effects on number of inflorescences and reproduction were mostly limited to 2014. Since treatments had minimal effects on pollinator visitation in 2014, the effect of clipping on plant reproduction may have been due to changes in resource availability rather than reductions in pollinator visitation.

Despite recurrent herbivory by cattle, the ability of clipped *Astragalus cicer L.* to produce similar amounts of vegetative biomass to unclipped plants may help to explain its abundance and success in the native grassland I studied. However, late grazing during flower development can negatively affect floral resources available to pollinators and plant reproduction, and can potentially lead to consequences for long-term fitness of *Astragalus cicer L.*

# Dedication

To my beloved family members, especially my mom,

whose wondrous home cooking I’ve missed dearly.

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# Table of Contents

Abstract ........ ii

Dedication ........ iv

Acknowledgements ........ v

Table of contents ........ vi

List of tables ........ viii

List of figures ........ ix

Chapter 1: General introduction ........ 1

    Plant-pollinator interactions ........ 1

    Grazing in grasslands ........ 2

    Effects of herbivory on growth, reproduction and pollination ........ 3

  Literature cited ........ 6

Chapter 2: Effect of timing and intensity of herbivory on growth and reproduction of an agronomic legume (*Astragalus cicer L.*) in the mixedgrass prairie ........ 12

  Introduction ........ 12

  Methods ........ 14

    Data analysis ........ 16

  Results ........ 17

    Growth ........ 17

    Reproduction ........ 17

  Discussion ........ 18

  Literature cited ........ 21

Chapter 3: Effect of timing and intensity of defoliation on floral traits and pollination in cicer milkvetch (*Astragalus cicer L.*) ........ 29

  Introduction ........ 29

  Methods ........ 32

    Pollinator observations ........ 33

    Nectar & sugars ........ 34

    Data analysis ........ 35

  Results ........ 37

    - Treatment and floral resources.......... 37
    - Floral resources and pollinator visitation.......... 38
    - Treatment effects and pollinator visitation on reproduction.......... 39
  - Discussion.......... 39
    - Intensity of clipping.......... 40
    - Timing of clipping and floral components.......... 40
    - Timing of clipping and plant reproduction.......... 41
    - Annual differences and abiotic factors.......... 42
    - Implications.......... 43
  - Literature cited.......... 43
- Chapter 4: Concluding remarks.......... 57
  - Literature cited.......... 60
- Full list of works cited.......... 63
- Appendices.......... 73
  - Appendix 1. Map of plant locations in field site.......... 73
  - Appendix 2. Regression of plant biomass VS initial plant size.......... 74
  - Appendix 3. Camera observations VS in person observations.......... 75
  - Appendix 4. Chapter 2 data: Differences to control plants.......... 76
  - Appendix 5. Chapter 3 floral data: Differences to control plants.......... 77
  - Appendix 6. Chapter 3 pollination data: Differences to control plants.......... 78
  - Appendix 7. Chapter 3 reproductive data: Differences to control plants.......... 79

## List of Tables

Table 2.1. ANOVA results for treatment effect on growth and reproduction .............................. 25
Table 3.1. ANOVA results for treatment effect on floral components......................................... 47
Table 3.2. ANOVA results for relationship between floral components and pollination............. 48
Table 3.3. ANOVA results for treatment effect on pollinator visitation ...................................... 49
Table 3.4. ANOVA results for relationship between floral components, pollination and reproduction ......................................................................................................................... 50
Table 3.5. ANOVA results for treatment effect on plant reproduction ........................................ 51

## List of Figures

Figure 2.1. Effect of treatment on growth........................................................................... 27
Figure 2.2. Effect of treatment on reproduction ............................................................... 28
Figure 3.1. Treatment effect on number of inflorescences and relationship between inflorescence numbers to pollinator visitation. ................................................................................... 52
Figure 3.2 Nectar sugars measured in 2015 ...................................................................... 53
Figure 3.3. Relationship between pollinator visitation and nectar sugars ................................... 54
Figure 3.4. Relationship between pollinator visitation and reproduction in 2014........................ 55
Figure 3.5. Measures of reproduction across clipping rimes for 2014 ........................................ 56

# Chapter 1: General Introduction

Excluding Antarctica and Greenland, grasslands cover as much as 40% of the earth (White et al. 2000) and Alberta contains nearly half of all Canadian grasslands (Bailey et al. 2010). Grasslands provide important ecosystem services such as carbon sequestration, pollination, areas for livestock to graze (Lamarque et al. 2011) and can provide key habitat for species at risk (Fish and Wildlife Division 2008). However, native grasslands have been continually lost through agricultural conversion into cropland and tame pasture. Grasslands play a key role in supporting pollinator populations by providing nesting habitat and a diversity of floral resources (Kells and Goulson 2003; Öckinger and Smith 2007; Potts et al. 2009), which is particularly important in the face of current global pollinator declines (Potts et al. 2010). When grasslands are grazed by livestock, grazers and pollinators may interact indirectly by foraging on the same plant (Gómez and Olivieri 2003; Mayer 2004; Vázquez and Simberloff 2004; Sjödin 2007). Changes in the abundance or composition of floral resources due to grazing may have consequences for pollinator communities and pollination services (Collins et al. 1998; Gómez and Olivieri 2003; Mu et al. 2016). Therefore, understanding species interactions in grassland systems is integral to grassland management and conservation of flora and fauna.

## *Plant-pollinator interactions*

Plants and pollinators share an intimate relationship, with many flowering plants relying on pollinators for sexual reproduction, and pollinators relying on plants for food. Approximately 85-90% of flowering plant species are animal pollinated (Kearns et al. 1998; Ollerton et al. 2011). Pollinators are attracted to plants which provide high amounts of floral resources through large numbers of flowers (Klinkhamer and de Jong 1990; Ohashi and Yahara 1998; Conner and Rush), or high amounts of nectar and pollen per flower (Thomson 1988; Klinkhamer and de Jong 1990; Mitchell 1994; Gómez et al. 2008). Flower sugar composition can also affect pollinator visitation as bumble bees and honey bees tend to prefer higher sucrose to hexose ratios (Wykes 1952; Perret et al. 2001). The relationship between pollinators and flowering plants is facing pressure from agricultural practices that lead to loss, fragmentation and degradation of floral resources found in native grasslands (Kearns et al. 1998). Declines in honey bee stocks in North America and Europe (Potts et al. 2010), and bumble bee declines in the UK (Biesmeijer et al. 2006) and North America (Cameron et al. 2011) could affect the diversity and abundance of wild plant species, as well as the production of agricultural crops. Invasive plants and escaped agronomics such as sweet clover and alfalfa may also offer high amounts of floral resources (Pedersen 1953; Furgala et al. 1958; Teuber et al. 1983; Chittka and Schürkens 2001), attracting native pollinators away from native plant species and displacing native plants over time (Brown and Mitchell 2001; Muñoz and Cavieres 2008).

### *Grazing in grasslands*

Many native grasslands also serve as rangelands, areas where large mammalian grazers forage. Grazers can moderate the functional and species diversity of plant communities by selectively foraging on certain species and changing ecosystem properties such as nutrient cycling (Collins et al. 1998; Díaz et al. 2007; Vilà et al. 2011). At the population level, grazers alter the abundance and spatial distribution of plants through the destruction of individuals, altering intra or inter-specific plant interactions or dispersal of seed via consumption (Noy-Meir et al. 1989; Adler et al. 2001; Vázquez and Simberloff 2004). At the individual level, grazing can affect plant growth, reproduction and survival (Hall et al. 1992; Trlica and Rittenhouse 1993). Plants may exhibit a variety of responses to grazing that alter plant growth and reproduction; they may suffer negative consequences, no consequences or overcompensate for the damage (Obeso 1993; Lennartsson et al. 1998; Hawkes and Sullivan 2001; Parra-Tabla et al. 2004). The putative benefits of herbivory has led to a plethora of research investigating the compensatory abilities of plants (Belsky 1986; Paige and Whitham 1987; Belsky et al. 1993; Agrawal et al. 2000). Much of existing research on plant compensation involves trade-offs between plant tolerance to herbivory and induced resistance to herbivory (Rosenthal and Kotanen 1994; Strauss and Agrawal 1999; Leimu and Koricheva 2006; Walters and Heil 2007), while a fewer number of studies have examined trade-offs between plant growth and reproduction following herbivory (Kozłowski 1992; Cheplick 1995; Schat and Blossey 2005).

### *Effects of herbivory on growth, reproduction and pollination*

When a plant is grazed and a portion of plant biomass is removed, it loses apical meristems, which may affect re-growth, nutrients stored in shoots, and the ability to acquire energy via photosynthesis due to reductions in total leaf area (Noy-Meir 1993). As such, some plants experience decreased shoot growth, stem biomass, leaf biomass and leaf area (Reichman and Smith 1991; Mauricio et al. 1993; Hickman and Hartnett 2002; Meyer and Hull-Sanders 2007). Other plants are able to tolerate relatively high levels of herbivory by maintaining or increasing growth rates (McNaughton 1979; Lennartsson et al. 1998; García and Mendoza 2012). The negative effects of herbivory on plant growth may be mitigated by activation of dormant meristems, increased light intensity to remaining leaves, and increased photosynthetic rates

(Noy-Meir 1993; Tiffin 2000). Plants can also respond to herbivory by re-allocating more root stores into regrowth (García and Mendoza 2012, Vilela et al. 2016).

Due to the high costs associated with plant reproduction (Pyke 1991; Obeso 1993), long-lived plants may sacrifice sexual reproduction within a growing season for increased leaf growth following herbivory to increase resource acquisition and storage for subsequent years (Simons et al. 1999; Hendrix 1984). Additionally, defoliation can reduce availability of resources which can lead to a reduced number of flowers and total reproductive biomass (Stamp 1984; Bergelson and Crawley 1992; Escarré et al. 1996; Hickman and Hartnett 2002; Spotswood et al. 2002). However, in response to herbivory, plants can allocate stored resources in roots to fruit and seed development (Mabry and Wayne 1997; Ida et al. 2012). Thus, despite losing up to 75% of leaf area, some plants suffer no reductions in reproductive biomass (Paige and Whitham 1987; Escarré et al. 1996; Mabry and Wayne 1997; Ida et al. 2012).

Herbivory can also alter plant reproduction through changes in flower number and floral traits, which could affect interactions with pollinators. Herbivory can lead to decreased flower number, flower display size, nectar guide area and nectar production, leading to negative effects on pollinator visitation (Strauss 1997; Mothershead and Marquis 2000; Suárez et al. 2009; Samocha and Sternberg 2010), although some studies show conflicting results (Halpern et al. 2010; Narbona and Dirzo 2010). Plants that have reduced flower number and nectar following herbivory may attract fewer pollinators, leading to lower reproductive success (Zimmerman 1983; Hodges 1995; Mothershead and Marquis 2000). Although the positive relationship between floral resource availability and pollinator visitation is well established (e.g. Quesada et al. 1995; Strauss et al. 1996; Gómez and Olivieri 2003), grazing can have indirect, mixed effects on pollinators. Moderate levels of grazing could have no effect (Vázquez and Simberloff 2004), increase (Mu et al. 2016) or decrease (Yoshihara et al. 2008) flower number and nectar amounts for pollinators and subsequently affect pollinator visitation. Although the effect of defoliation intensity on plant pollination and reproduction has been fairly well studied (Krupnick et al. 1999; Mothershead and Marquis 2000; Hudewenz et al. 2012), there is a paucity of studies on how timing of defoliation and interactions between timing and intensity, affect plant pollination and reproduction.

In my first chapter, I investigate how timing and intensity of herbivory affect plant growth and reproduction by clipping plants and measuring end of season biomass to different plant parts. I hypothesized that higher levels of defoliation and defoliation earlier in the season leads decreased production of reproductive relative to vegetative biomass. In chapter 2, I explore how timing and intensity of herbivory affect floral components, pollinator visitation and plant reproduction. I hypothesized that a higher level of defoliation and defoliation earlier in the season would decrease floral resources produced, negatively affecting plant pollination and reproduction. My focal study species is cicer milkvetch (*Astragalus cicer L.*), a large, non-native forage crop introduced from Europe. Cicer milkvetch, is spreading into native grasslands and produces a massive floral display which attracts both native and non-native pollinators. Understanding how grazing affects cicer milkvetch and its relationship with pollinators will help manage this species, which is an important forage species for cattle, a food source for pollinators and a potential competitor with native grassland plants.

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# Chapter 2: Effect of timing and intensity of herbivory on growth and reproduction of an agronomic legume (*Astragalus cicer L.*) in the mixedgrass prairie

## Introduction

Herbivory can cause trade-offs between plant growth and reproduction leading to changes in survival and fitness. After herbivory, initial energy investments to plant structures are lost, resulting in reduced photosynthetic capacity and potentially reduced total biomass production and reproductive output (Jameson 1963; Strauss and Agrawal 1999; Hickman and Hartnett 2002). In order to increase fitness following herbivory, perennial plants may allocate resources towards vegetative growth to increase photosynthesis, but sacrifice sexual reproduction for the season (Bazzaz et al. 1987; Garnier 1992). Even though perennial plants lose out on sexual reproduction for the year, they may be able to reproduce in subsequent seasons, thus, potentially increasing their fitness over the long-term by investing in growth, a form of bet-hedging (Simons et al. 1999; Brown and McNeil 2006). The effect of herbivory on the growth and reproduction of perennial forbs varies, with some species demonstrating reductions in growth or reproductive output following herbivory, while others are not affected (Bentley et al. 1980; Paige and Whitham 1987; Agren 1989; Obeso 1993). Part of this variation in plant response to herbivory is likely influenced by the timing and intensity of herbivory.

At high levels of defoliation, plants may suffer substantial negative consequences such as decreased shoot biomass, reproduction and root growth, but at low to moderate levels of defoliation, some plants experience no changes in growth or reproduction (Hickman and Hartnett 2002; Hong et al. 2003; García and Mendoza 2012). The effects of low or moderate levels of defoliation may be countered by increases in photosynthetic rates due to increased leaf growth, delayed leaf senescence, and less self-shading (Brougham 1956; Nowak and Caldwell 1984; Tiffin 2000; García and Mendoza 2012). Further, plants can re-allocate more root stores to maintain their growth at higher intensities of herbivory (García and Mendoza 2012; Vilela et al. 2016). Timing of herbivory can also influence the magnitude and direction of plant response to herbivory. Early in the growing season, resources from root stores are used towards aboveground growth to enable photosynthesis, while later in the growing season, more resources are acquired and this can be allocated, in part, towards reproduction (Willson 1983; Swanton and Cavers 1989; Machado et al. 2013). Thus, timing of herbivory can have a strong impact on the production of reproductive biomass. For example, herbivory that occurs after flowering in forbs can have a negative effect on subsequent floral and fruit development (Thomson et al. 2002; Anderson and Frank 2003). The intensity of defoliation can interact with timing, magnifying negative effects of defoliation and affecting future growth and reproduction. For example, a higher intensity of defoliation can have a greater negative effect on growth and reproduction after plants begin to flower (Gedge and Maun 1992; Gregorutti et al. 2012; Sharma 2013). Perennial forbs have a variable response to herbivory (Obeso 1993); studying how timing and intensity of herbivory can affect perennials in agroecosystems can have direct implications for management strategies.

Cicer milkvetch (*Astragalus cicer* L.) is a large perennial legume introduced to North America from Europe as forage for cattle and is commonly used in pasture (Acharya et al. 2006). While the vegetative response of cicer milkvetch to grazing in hayfields has been studied (Smoliak and Hanna 1975; Townsend et al. 1978; Kephart et al. 1990), effects of grazing on cicer milkvetch have not been studied in native grasslands. In native grasslands, cicer may be a valuable forage for cattle, but it may also compete with native plant species for resources.

Depending on management goals, ranch mangers may want to maintain cicer milkvetch in rangelands as forage or reduce its growth if cicer is negatively affecting other native species. Thus, I investigated the response of cicer milkvetch to clipping to examine how the timing and intensity of grazing might affect its growth and reproduction. I predicted higher levels of clipping and clipping earlier in the season would lead to increased vegetative growth and decreased fruit production in grassland populations of *A. cicer*. In order to compare plant growth and reproduction, I measured the production of vegetative and reproductive biomass. Understanding the response of cicer milkvetch to these treatments could enable the use of grazing as a management tool to control the plant in the community or inform management to help promote plant growth and reproduction.

## Methods

I conducted this study at the University of Alberta's Mattheis Research Ranch near Duchess, Alberta, Canada (50°53'N; 111°52'W) which lies within the Dry Mixedgrass Prairie Natural Subregion (Downing and Pettapiece 2006). Dominant grass species included Needle-and-thread grass (*Hesperostipa comata* (Trin. & Rupr.) Barkworth), Prairie sandreed *(Calamovilfa longifolia* (Hook.) Hack. ex Scribn. & Southw.), and Blue Grama (*Bouteloua gracilis* (Kunth) Lag. ex Griffiths). During the two growing seasons (April to September) of this study, 274 mm and 194 mm of precipitation fell in 2014 and 2015, respectively. During the 2014 and 2015 growing season, mean temperatures were 13.4 °C and 14 °C, respectively (Alberta Agriculture and Forestry Statistics for Verger, Alberta; located approximately 1 km from study site).

In 2014, I selected 60 plants dispersed across a 1.5 km$^2$ area on Mattheis ranch (GPS coordinates of plants in Appendix 1). I selected plants up to a size of two meters in diameter and plant area ranged from 0.5 m$^2$ to 4 m$^2$. Plants were at least 30 m apart and were randomly assigned to one of five treatments. In a factorial design, I manipulated the timing (early and late) and intensity (low and high) of defoliation by hand clipping. The fifth treatment was a control in which these plants were never clipped. Early clipping occurred when plants started to form buds (June 20-22 in 2014 and June 8-11 in 2015) and late clipping occurred two weeks later, prior to flowering. I visually estimated clipping intensity, removing a third of every stem on plants in the low intensity treatment and removing two-thirds of every stem on the plants in the high intensity treatment. These grasslands are grazed once per season in a short duration rotation through the rangeland. Individual plants received the same treatment in both 2014 and 2015. Cages were placed over all experimental plants to prevent cattle from grazing them.

To examine plant response to clipping, I measured a number of plant traits important to growth, development and fitness. I counted the total number of stems, measured the stem length of 10 random stems, and measured plant spread to determine plant growth prior to the first clipping and eight weeks after the final clipping in both years. Plant spread was calculated by measuring the length of the longest axis of cover and the length of the axis perpendicular to it, then multiplying the two measurements. At the end of the season, I collected five basal flowering stems from each plant to estimate leaf, stem and fruit biomass. All plant samples were dried at 60 °C for at least 48 hrs before being weighed. Plant-level response was estimated by multiplying data from the sub-sample of stems with the final stem count for each plant at the end of the season. Total reproductive biomass was the sum of all fruit biomass at the end of the season, while vegetative biomass included both leaf and stem biomass.

*Data analysis*

All analyses were completed in R (version 3.3.1). Data were log transformed as needed to approximate a normal distribution. Differences in growth and reproduction between each of the four clipping treatments and control plants was analyzed with the Dunnett's test, which allows multiple comparisons to a control. First I constructed a linear mixed model with treatment and initial plant size as fixed effects and individual plants as random effects using the lme4 package (Bates et al. 2015). Then, I applied a Dunnett's test using the multcomp package (Hothorn et al. 2008). In a separate model, I tested the interaction between timing and intensity of clipping, where plant growth metrics, plant biomass and reproductive variables were analyzed in a linear mixed model with year, timing of clipping, intensity of clipping, and initial plant size as fixed effects and individual plant as a random effect. Initial plant size was calculated by multiplying average stem length by total number of stems prior to any clipping in the first summer. This index was found to correlate highly with plant biomass (Appendix 2). There was no difference in initial plant size ($F_{4,49} = 0.30$, $p = 0.585$) or the initial area ($F_{4,49} = 0.05$, $p = 0.891$) of plants assigned to the different treatments. When comparing differences in plant spread and stem count before and after clipping, initial plant size was excluded from the model. I evaluated the models with a Type III F-test using the car package (Fox and Weisberg 2010). When models were significant, I ran post-hoc comparisons using Tukey tests with the lsmeans package to distinguish which treatments were significantly different from each other (Lenth 2016).

## Results

### *Growth*

Clipping affected vegetative and reproductive measures when each treatment was compared to control plants (Appendix 4). Compared to control plants, clipped plants on average experienced decreased plant spread over the growing season, decreased vegetative biomass, stem biomass, and shoot growth, but increased ratio of leaves to stems. Clipped plants were not different from control plants in terms of stem count and leaf biomass (Appendix 4). Of the variables affected by clipping, timing and intensity of clipping affected change in plant spread, shoot growth, vegetative biomass, and stem biomass (Table 2.1A & B). Change in spread, from before to after clipping, was different between clipping intensities and between years (Table 2.1). High clipping intensity led to a reduced change in spread compared to control plants (Fig 2.1A). Change in plant spread, from before to after clipping, was greater in 2015 compared to 2014 (Fig 2.1B). Stem growth was affected by an interaction between clipping intensity and year, while timing of clipping had no effect on stem growth (Table 2.1A & B). High intensity clipping increased stem growth in 2014, while in 2015, clipped plants on average had decreased stem growth relative to control plants (Fig. 2.1C). Total vegetative biomass was affected by an interaction between timing, intensity and year (Table 2.2B). Late-high intensity clipping treatments had less vegetative biomass in 2014 compared to 2015, but there were no significant differences within each year (Fig 2.1D). Although stem biomass had a significant three-way interaction, a post hoc test did not reveal differences between treatments.

### *Reproduction*

Clipped plants experienced decreases in reproductive measures compared to control plants (Appendix 4). Total fruit biomass was affected by a three-way interaction between timing, intensity and year, but only the main effect of timing was significant (Table 2.1A & B). In 2014, late-high intensity clipping had lower fruit biomass and fewer fruits compared to early clipping treatments, and early-high had higher fruit biomass compared to late-low clipping (Fig. 2.2A). However, fruit biomass was not significantly different between treatments in 2015 (Fig. 2.2A). Ratio of fruit to vegetative (leaf and stem) biomass was affected by an interaction between timing and year, with timing as a significant main effect (Table 2.1A & B). In 2014, late clipping led to a lower ratio of fruits to vegetative biomass compared to early clipping and control plants (Fig 2.2B), but in 2015, there was no difference due to timing of clipping.

## Discussion

Herbivory can be costly for plant growth and reproduction and the magnitude of these costs can change based on when and how much damage is incurred. Thus, managing the timing and intensity of grazing may affect the vegetative growth and reproduction of cicer milkvetch. However, contrary to my predictions, I did not find that higher clipping intensity or earlier clipping led to more vegetative growth compared to fruit production. Instead, I found that **1)** my clipping treatments had minimal effects on plant growth, **2)** both timing and intensity affected fruit production, with late-high intensity clipping having the strongest negative impact on fruit production, and **3)** the first year of clipping had a stronger negative effect on reproduction compared to the second year.

The production of vegetative biomass by cicer milkvetch was largely unaffected by the timing and intensity of clipping. However, a higher intensity clipping reduced plant spread overall. In the second year, there was an increase in spread which may have been due to second year effects of clipping. An overall reduction in plant spread may be due to increased stem branching under higher intensity grazing (Escarré et al. 1996), whereas a greater increase in spread in the second year may due to increased lateral rather than vertical stem growth to tolerate grazing (McIntyre and Lavorel 2001). Although a high intensity clipping led to a decrease in plant spread overall, plants may not necessarily suffer decreased growth under high intensity clipping, since, in 2014, I observed that a high intensity clipping led to higher stem growth compared to low intensity clipping. Growth of the perennial herb *Convolvulus demissus* was also unaffected by herbivory as 50% defoliation had no effect on number of leaves and shoot biomass and even increased total number of stems and total plant biomass (Quezada and Gianoli 2010). Although, defoliation led to lower above ground biomass in lima bean (*Phaseolus lunatus*), there were no differences between high and low levels of defoliation (Blue et al. 2015). Similarly, we found clipping affected vegetative growth of cicer, but clipping intensity had minimal effects on growth. Clipping time also had minimal effects on vegetative growth in cicer milkvetch, nor did it affect the production of vegetative biomass of the biennial/perennial herb *Picris hieracioides* (Escarré et al. 1996). Plants may be able to regrow following herbivory through increased photosynthetic rates after damage, especially when subjected to higher intensities of herbivory (Brougham 1956; Meyer 1998; Tiffin 2000; Thomson et al. 2002). Future studies on response of cicer milkvetch following herbivory could determine whether it also employs these mechanisms.

Cicer milkvetch demonstrated reduced reproductive output after clipping, but this effect is smaller when defoliation occurs earlier in the flowering period. Considering the sizeable cost of reproduction for plants (Southwick 1984; Pyke 1991; Obeso 2002), clipping during a late stage in flower development could have particularly negative effects on flowering and fruit mass (Michaud 1991; García and Ehrlén 2002), perhaps due to a shorter window for regrowth following herbivory. Indeed, decreased fruit to vegetative biomass ratio (in 2014) in late season clipping treatments relative to early season clipping show that late clipping has a greater negative impact on plant reproduction compared to early clipping.

Although a large proportion of clipping studies on non-grasses demonstrate negative effects on reproduction when plants were clipped several weeks prior to flowering (Anderson and Frank 2003), I observed no effects on seed production relative to control plants when plants were clipped when buds first formed, approximately 3 weeks prior to flowering. However, I found negative effects on reproduction when plants were clipped at high intensity two weeks after bud formation. Some studies on perennial plants have shown that higher clipping intensities can negatively affect reproductive biomass (Doak 1992; Hickman and Hartnett 2002), while other studies showed little effect of clipping intensity on perennial plant reproduction (Agren 1989; Lubbers and Lechowicz 1989). There was a greater negative effect on total fruit production of lima bean (*Phaseolus lunatus L.*) when the plants were continuously defoliated at 66% defoliation compared to 33% defoliation (Blue et al. 2015). In a similar experiment where mung beans (*Vigna radiata*) were clipped only once, increased clipping intensity had a stronger negative effect on fruit production when plants were clipped at the flowering stage, while increased clipping intensity had a weaker negative effect when plants were clipped 10 days prior to flowering (Sharma 2013). I also found a negative effect of late clipping on fruit production and fruit allocation, but only in the first year of study. Cicer milkvetch reproduction is affected by timing and intensity of herbivory, but other factors such as resource availability should also be considered (Maschinski and Whitham 1989), since initial plant size also affected plant growth and reproduction (Table 2.1A).

Perennial plant growth and reproduction can vary significantly year to year (Doak 1992), however; consecutive years of herbivory for perennial plants may compound the effects of defoliation on plant growth and reproduction, as seen in the perennial herb *Sanicula europaea* (Gustafsson 2004). Despite this, I found that cicer milkvetch plants with late high intensity defoliation had a lower reproductive output in 2014, but increased reproduction in 2015. The mechanism driving this difference between treatment years is not known, but could be related to differences in resource allocation between years or to variation in environmental conditions. Given that I generally observed a negative response in the first year of my clipping treatments, longer, multi-year studies are needed to understand cicer milkvetch's response to herbivory.

Although under grazing pressure from cattle, cicer milkvetch is a common and resilient plant in our study area within native grasslands. My study suggests that cicer milkvetch can sustain high intensities of grazing with limited impact on vegetative growth. However, higher intensity grazing once flower bud are well-developed can negatively affect fruit production. In order to maintain cicer milkvetch in the rangelands, ranch managers should plan to graze prior to bud development, or at an early stage in bud development to minimize impact on plant reproduction. Cicer milkvetch displays a high tolerance to herbivory, which makes it well-suited as a forage crop in regularly grazed rangelands.

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## Tables

Table 2.1A. ANOVA results for Treatment Effect on Growth and Reproduction. F-test results showing variables from a generalized linear mixed model that significantly affected plant traits relating to growth and reproduction of cicer milkvetch. I manipulated timing and intensity of clipping, following the same plants over two summers.

| | **Timing** | | | **Intensity** | | | **Year** | | | **Initial plant size** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Growth** | F-value | df | **p-value** | F-value | df | **p-value** | F-value | df | **p-value** | F-value | df | **p-value** |
| Change in stem count | 0.05 | 1, 77.9 | 0.816 | 0.01 | 1, 77.9 | 0.917 | 0.34 | 1, 39 | 0.564 | NA | NA | NA |
| Change in spread ($m^2$) | 0.74 | 1, 78 | 0.392 | 3.99 | 1, 78 | **0.049** | 14.78 | 1, 39 | **<0.001** | NA | NA | NA |
| Shoot growth (cm) | 0.09 | 1, 39 | 0.762 | 9.69 | 1, 39 | **0.003** | 0.46 | 1, 39 | 0.501 | 0.36 | 1, 38 | 0.553 |
| Vegetative biomass (g) | 4.98 | 1, 66.9 | **0.029** | 0.50 | 1, 67.5 | 0.483 | 0.04 | 1, 38.1 | 0.841 | 27.65 | 1, 38 | **<0.001** |
| Leaf biomass(g) | 4.51 | 1, 38.1 | **0.040** | 0.98 | 1, 39.2 | 0.329 | 1.98 | 1, 38.1 | 0.168 | 23.37 | 1, 37.9 | **<0.001** |
| Stem biomass (g) | 4.24 | 1, 38.1 | **0.046** | 1.46 | 1, 39 | 0.235 | 1.75 | 1, 38.1 | 0.194 | 16.98 | 1, 38 | **<0.001** |
| Leaves : stem biomass | 0.26 | 1, 38.2 | 0.612 | 1.22 | 1, 39.2 | 0.277 | 18.75 | 1, 38.2 | **<0.001** | 0.18 | 1, 37.9 | 0.676 |
| **Reproduction** | | | | | | | | | | | | |
| Fruit biomass (g) | 30.75 | 1, 37.6 | **<0.001** | 1.11 | 1, 38.1 | 0.298 | 2.11 | 1, 38.1 | 0.155 | 9.03 | 1, 37.8 | **0.005** |
| Fruits: veg. biomass | 20.35 | 1, 36.5 | **<0.001** | 1.73 | 1, 36.6 | 0.196 | 16.50 | 1, 36.5 | **<0.001** | 1.52 | 1, 37.4 | 0.225 |

Table 2.1B (cont'd). ANOVA results for treatment effect on growth and reproduction. F-test results showing variables from a generalized linear mixed model that significantly affected plant traits relating to growth and reproduction of cicer milkvetch. I manipulated timing and intensity of clipping, following the same plants over two summers.

| | **Timing x Intensity** | | | **Timing x Year** | | | **Intensity x Year** | | | **Timing x Intensity x Year** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Growth** | F-value | df | **p-value** | F-value | df | **p-value** | F-value | df | **p-value** | F-value | df | **p-value** |
| Change in stem count | 0.11 | 1, 77.9 | 0.747 | 0.17 | 1, 39 | 0.682 | 0.74 | 1, 39 | 0.394 | 2.61 | 1, 39 | 0.115 |
| Change in spread ($m^2$) | 0.31 | 1, 78 | 0.582 | 1.92 | 1, 39 | 0.174 | 0.14 | 1, 39 | 0.706 | 1.78 | 1, 39 | 0.189 |
| Shoot growth (cm) | 2.52 | 1, 39 | 0.120 | 0.09 | 1, 39 | 0.761 | 9.68 | 1, 39 | **0.003** | 2.52 | 1, 39 | 0.120 |
| Vegetative biomass (g) | 1.62 | 1, 66.8 | 0.207 | 5.16 | 1, 38.1 | 0.029 | 1.70 | 1, 39.1 | 0.199 | 8.67 | 1, 38.6 | **0.005** |
| Leaf biomass (g) | 8.24 | 1, 38.7 | **0.007** | 4.51 | 1, 38.1 | **0.040** | 0.98 | 1, 39.2 | 0.329 | 8.24 | 1, 38.7 | **0.007** |
| Stem biomass (g) | 4.58 | 1, 38.6 | **0.039** | 4.24 | 1, 38.1 | 0.046 | 1.45 | 1, 39 | 0.235 | 4.58 | 1, 38.6 | **0.039** |
| leaves: stem biomass | 0.22 | 1, 38.7 | 0.643 | 0.26 | 1, 38.2 | 0.612 | 1.22 | 1, 39.2 | 0.276 | 0.22 | 1, 38.7 | 0.643 |
| **Reproduction** | | | | | | | | | | | | |
| Fruit biomass (g) | 10.10 | 1, 37.6 | **0.003** | 30.73 | 1, 37.6 | **<0.001** | 1.11 | 1, 38.1 | 0.298 | 10.09 | 1, 37.6 | **0.003** |
| Fruits: veg. biomass | 0.54 | 1, 36.5 | 0.469 | 20.33 | 1, 36.5 | **<0.001** | 1.73 | 1, 36.6 | 0.196 | 0.54 | 1, 36.5 | 0.469 |

## Figures

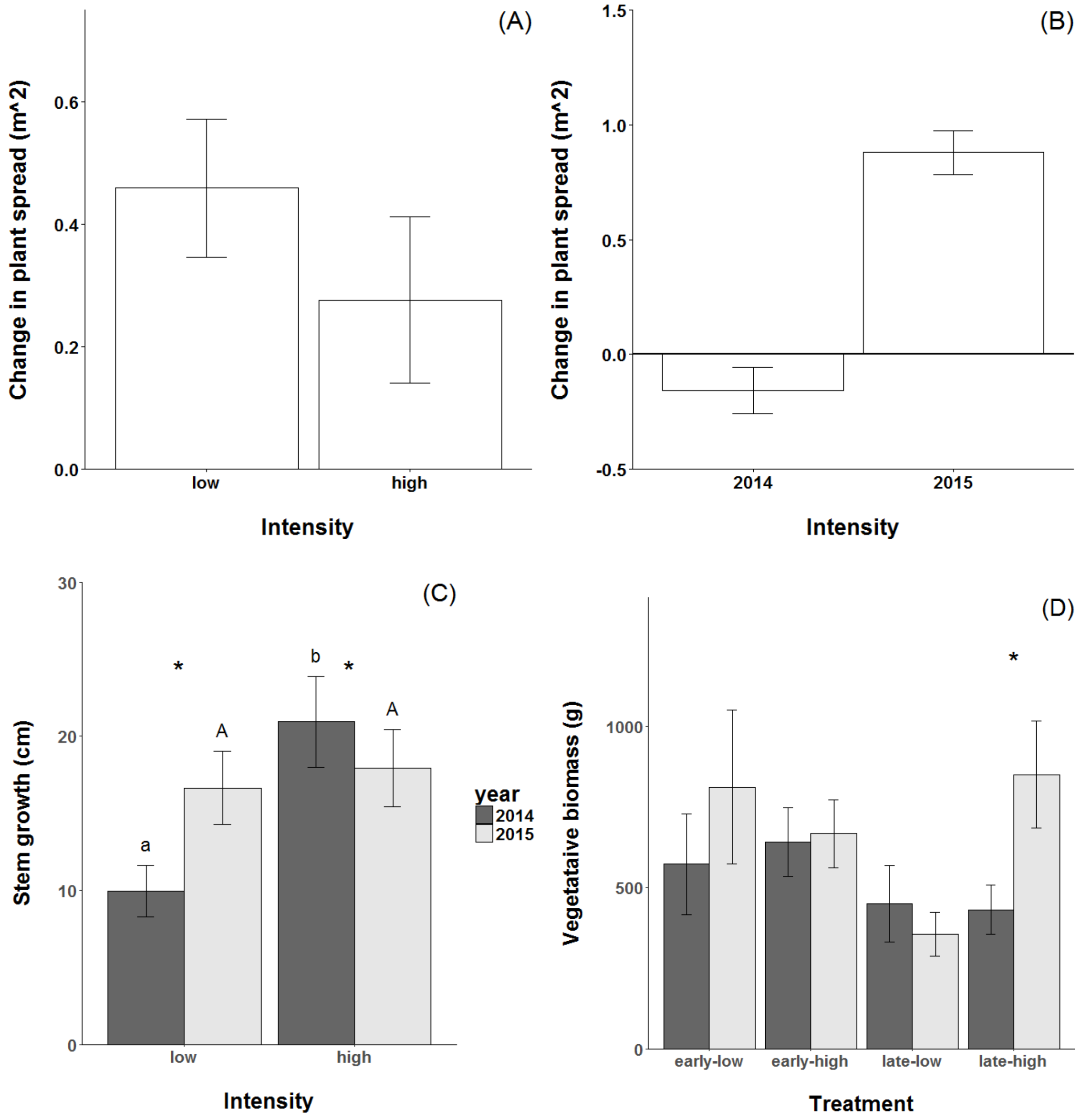

Figure 2.1. Effect of treatment on growth. Mean (+/- SE) of plant growth measurements for A) change in plant spread across clipping intensity, B) change in plant spread over 2 years C) stem growth over 8 weeks, and D) total vegetative biomass by treatment and when significantly different, by year. Significant differences between groups (Tukey test, P<0.05) in 2014 and 2015 are represented by lower case letters and upper case letters, respectively. An asterisk indicates significant treatment differences between 2014 and 2015 (Tukey test, P<0.05).

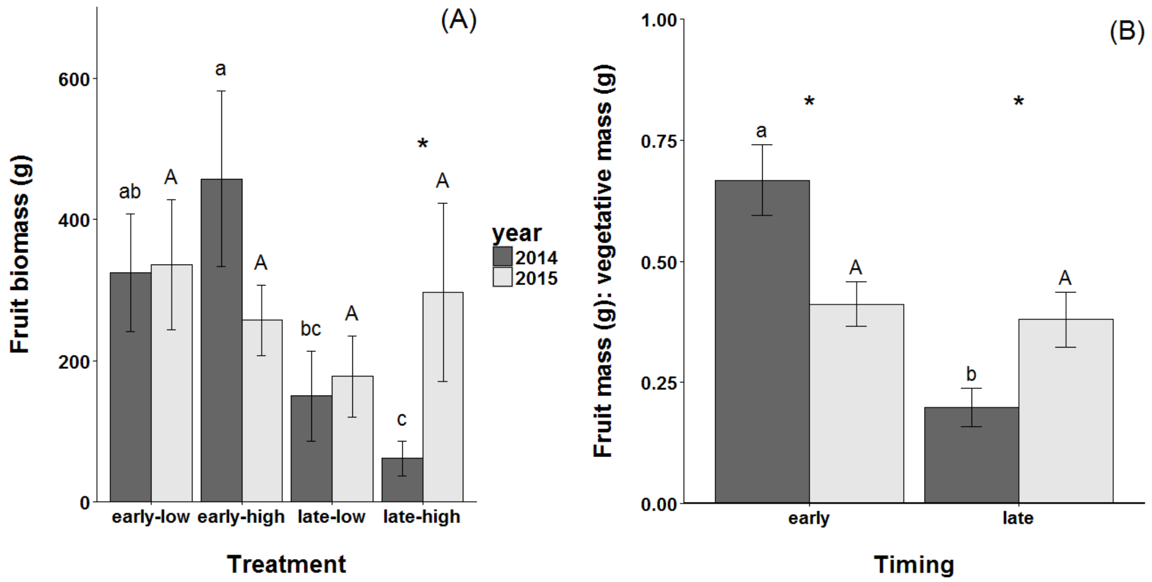

Figure 2.2. Effect of treatment on reproduction. Mean (+/- SE) of plant reproduction measurements for A) Fruit biomass B) fruit/vegetative biomass by treatment and by year. Significant differences between groups (Tukey test, P<0.05) in 2014 and 2015 are represented by lower case letters and upper case letters, respectively. An asterisk indicates significant treatment differences between 2014 and 2015 (Tukey test, P<0.05).

# Chapter 3: Effect of timing and intensity of defoliation on floral traits and pollination in cicer milkvetch (*Astragalus cicer L.*)

## Introduction

Plant responses to herbivory may include changes in allocation to floral resources that could affect their visitation by pollinators and subsequent reproduction (Gómez and Olivieri 2003; Mayer 2004; Vázquez and Simberloff 2004; Sjödin 2007). Reproduction is a costly investment for plants; thus, when plants are consumed by herbivores (Bazzaz et al. 1987; Primack and Hall 1990; Garnier 1992; Obeso 2002), plants may allocate their remaining resources towards growth to recapture more resources instead. Pollinator visitation is generally driven by flower number and nectar rewards, which can affect plant reproductive success (Klinkhamer and de Jong 1990; Kunin 1993; Conner and Rush 1995; Ohashi and Yahara 1998; Knight 2003). After herbivory, there may be a reduction in the total number of flowers per plant, and the associated pollen and nectar available to pollinators, leading to declines in pollinator visitation rates and seed production (Quesada et al. 1995; Strauss et al. 1996; Gómez and Olivieri 2003). Non-floral herbivory can also indirectly affect nectar production (Samocha and Sternberg 2010), and subsequently pollinator visitation. Furthermore, to my knowledge, no studies have examined the effect of herbivory on sugar composition in nectar, which could lead to differences in the numbers and types of pollinators that visit (Wykes 1952; Perret et al. 2001). The effect of herbivory on floral resources, plant pollination and reproduction may be influenced by both the intensity and timing of herbivory.

Intensity of herbivory, the amount of plant biomass removed, can influence both the allocation of remaining resources and the amount of photosynthetic tissue available to acquire energy following herbivory. After herbivory, stored resources can be directed towards regrowth of leaves which may reduce resource allocation that would have been directed to reproduction (García and Mendoza 2012). Intensity of defoliation can also affect inflorescence production for multiple years (Vilela et al. 2016), and decrease nectar production with increasing herbivory (Ida et al. 2012; Blue et al. 2015). Reduced floral resources could lead to decreased visitation rates with increasing herbivory. However, the relationship between intensity of herbivory and nectar production may not always be linear, as low to moderate levels of grazing have been shown to increase nectar production in plants relative to plots that are ungrazed or grazed at high intensities (Mu et al. 2016).

Timing of herbivory can have different effects on plant resource allocation depending on the level of reproductive investment prior to flowering. Plants typically accumulate maximal carbohydrate reserves prior to flowering, and reserves are reduced as plants produce flowers and seeds (Donart and Cook 1970; Menke and Trlica 1981). When plants are investing their resources into reproduction, herbivory can have a strong impact on the production of reproductive biomass. For example, the later that spotted knapweed (*Centaurea stoebe L.*) was clipped in the growing season, the lower the number of buds and flowers produced (Benzel et al. 2009). Further, defoliation of emergent shoots of fireweed (*Epilobium angustifolium L.)* had no effect on vegetative growth or flower production, while defoliation just prior to flowering significantly affected flower production (Michaud 1991). There are many studies demonstrating negative impacts of herbivory on floral traits and pollination (Strauss et al. 1996; Lehtilä and Strauss 1997; Mothershead and Marquis 2000; Hambäck 2001; Parra-Tabla and Herrera 2010);

however, few studies have examined the effects of both timing and intensity of herbivory on floral investment and subsequent pollination.

In this study, I tested the effects of the timing and intensity of defoliation on the pollinator visitation and reproduction of cicer milkvetch (*Astragalus cice*r L.) in the mixedgrass prairie. Cicer milkvetch is a large perennial legume introduced to North America from Europe as forage for cattle that is spreading into native grasslands. These plants grow in dense patches and produce a large floral display, attracting both native and managed pollinators (Richards 1987). Although cicer milkvetch is capable of self-pollinating, it greatly benefits from pollination, as only 2% of non-pollinated flowers set seed (Richards 1986). Studies have been conducted on pollination of cicer milkvetch in pastures and plant nurseries, but no studies have investigated pollination of cicer in native grasslands. I simulated cattle grazing to examine how the timing and intensity of grazing influences allocation to floral resources, pollinator visitation and reproductive output of cicer milkvetch**.** I addressed 3 questions: first does the timing and intensity of clipping affect production of floral resources? Second, if so, does this affect the visitation rate by pollinators to the plant? And finally, does the resulting variation in pollinator visitation affect reproductive output? I predicted higher levels of defoliation and defoliation earlier in the season would lead to decreased investment to floral components, decreased pollination rates and subsequent reproduction in *A. cicer*. To study effects of clipping on floral components and pollination, I measure the production of flowers, nectar, fruits, and seed set and record pollinator visitation rates. My research will reveal whether the direct effects of defoliation or the indirect effects on pollinator visitation due to defoliation affect plant reproduction and whether this relationship is mediated by timing and intensity of defoliation.

## Methods

I conducted this study at the University of Alberta's Mattheis Research Ranch near Duchess, Alberta, Canada (50°53'N; 111°52'W) which lies within the Dry Mixedgrass Prairie Natural Subregion (Downing and Pettapiece, 2006). The vegetation is dominated by grasses such as *Calamovilfa longifolia* ((Hook.) Hack. ex Scribn. & Southw.)*, Hesperostipa comate* ((Trin. & Rupr.) Barkworth)*,* and forbs such as *Dalea purpurea* (Vent.), *Vicia americana* (Muhl. ex Willd), *Astragalus agrestis* (Douglas ex G. Don), and *Cirsium flodmanii* ((Rydb.) Arthur). During the study, 274 mm and 194 mm of precipitation fell during the growing season (April to September) in 2014 and 2015, respectively. During the 2014 and 2015 growing season, mean temperatures were 13.4 °C and 14 °C, respectively (Alberta Agriculture and Forestry Statistics for Verger, Alberta; located approximately 1 km from study site).

In 2014, I selected 60 plants dispersed across a 1.5 $km^2$ area on Mattheis ranch (GPS coordinates of plants in Appendix 1). I selected plants up to a size of two meters in diameter and plant area ranged from 0.5 $m^2$ to 4 $m^2$. Plants were at least 30 m apart and randomly assigned to one of five treatments. In a factorial design, I manipulated the timing (early and late) and intensity (low and high) of defoliation by hand clipping. The fifth treatment was a control, where plants were never clipped. Early clipping occurred when plants just started to form buds (June 20-22 in 2014 and June 8-11 in 2015) and late clipping occurred two weeks later, prior to flowering. Plants in the low clipping intensity treatment had a third of every stem on the plant removed, while the high intensity clipping removed two-thirds of every stem on the plant; these amounts were visually estimated. Individual plants received the same treatment in both 2014 and 2015. Cages were placed over all plants to prevent cattle or other wildlife from grazing the plants.

To examine plant allocation towards reproductive traits and plant reproductive success, I measured inflorescence, fruit and seed production each year. When fruits had matured, I collected five basal stems from each plant to estimate the ratio of fruit to vegetative biomass, number of fruits produced per inflorescence, individual seed mass, and seed yield. From the fruited inflorescences, I estimated number of flowering inflorescences since both measures were highly correlated. Fruits were counted on a sample of 10 inflorescences and seeds were separated from other plant material with 1mm sieves and weighed (no seeds were larger than 1 mm). All plant samples were dried at 60 °C for at least 48 hrs before being weighed. Reproductive success for each plant was estimated by multiplying the data collected on subset of stems with a final stem count at the end of the season. To account for plant variation in size at the beginning of the experiment, I counted the total number of stems and measured stem length prior to any defoliation.

### *Pollinator observations*

I conducted pollinator observations on all 60 plants in both study years. Observations were only conducted when the temperature was above 20°C, wind speed was below 20 km/hr and when it wasn't raining. Observations were conducted between 7 am and 7 pm on three separate days. Although pollinator observations for each plant occurred on different days, I attempted to visit plants at least once in the morning/afternoon (7am-1pm) and at least once in the late-afternoon/evening (1pm - 7pm) to capture some temporal variation in pollinator activity. In 2014, from July 15th to August 1st over 8 days, pollinator observations were conducted in person, with a 15-minute observation period per plant on three different days, resulting in 45 mins of total observation time per plant. To increase my observation time and power to detect treatment effects, in 2015, I monitored pollinators using video cameras. From July 3rd to July 22nd, pollinator observations were conducted at each plant using a small HD video camera (Mobius Actioncam) which were affordable, light, provided high resolution and had a range of configurable settings. I hammered wooden stakes to the ground approximately 30 cm from each plant and attached a video camera with an external battery pack using a small tripod (PEDCO) with a Velcro strap. Video observations followed a similar protocol to 2014 such that each plant was recorded at least once in the morning/afternoon, and at least once in the late-afternoon/evening. I recorded each plant three times for 30 minutes, which resulted in 90 minutes of video footage per plant. I recorded the total number of inflorescences visited, the total time spent visiting and the type of pollinator that visited; visits were defined as instances when pollinators touched an inflorescence. In both years, I identified pollinators into two functionally important categories: *Bombus* and Apidae. Although eight different genera of pollinators have been observed to visit cicer milkvetch in Lethbridge (Richards 1986), *Bombus* are known to be the most efficient pollinators of cicer (Richards 1987) and made up around half the visits in my study. Potential differences between in person observation and camera observations were not tested, and as a result, each year of pollinator observations are analyzed separately. Further discussion on these two methods is presented in Appendix 3.

### *Nectar & sugars*

To estimate production of floral resources for pollinators, a key component of reproduction, I measured nectar volume and nectar sugar mass. Nectar was collected between July 9th and July 23rd in 2015, overlapping with my pollinator observations. In 2015, I collected nectar samples on 10 plants per treatment. The night before nectar collection, I bagged five inflorescences on each plant with tulle netting to prevent nectar loss to pollinators; I then collected nectar from five flowers per inflorescence using 2 μL microcapillary tubes and recorded nectar volume per flower. All nectar collection was done at dawn to minimize evaporative water loss. Nectar from each plant, representing a total of 25 flowers, was aggregated into one sample, which was diluted in 500μL of 70% ethanol and stored at -18°C. Samples were later analyzed for fructose, glucose and sucrose content using HPLC (Varian Prostar) with an Evaporative Light Scattering Detector (ELSD; Alltech 3300) and a 25cm x 4.6mm, 55μm column for separating sugars (SUPELCOSIL$^{TM}$ LC-NH2). The mobile phases consisted of water and acetonitrile, set at a flow rate of 1.5 mL/min. For ELSD detection, nitrogen gas flow rate was set to 3 standard litres per minute (SLPM) and drift temperature set to 110 degrees C. Eight standards of different sugar concentrations containing a mixture of fructose, glucose and sucrose were run between samples for calibration. I used Galaxie software v 1.9.302.952 (Agilent Technologies, discontinued) to calculate the total concentration of individual sugars in each sample.

### *Data analysis*

I analyzed each year of all data separately due to the different methods used to observe pollinators, with all analyses conducted in R (version 3.3.1). I analyzed the effect of treatment on floral traits, pollination and reproduction using a generalized linear model. Differences in between each of the four clipping treatments and control plants was analyzed with the Dunnett's test, which allows multiple comparisons to a control treatment. First, I constructed a generalized linear model with treatment and initial plant size as explanatory variables, and then, I applied a Dunnett's test using the multcomp package (Hothorn et al. 2008). Initial plant size was estimated by multiplying stem number by stem length and correlated highly with plant biomass (Appendix 2). Neither initial plant size ($F_{4,49} = 0.30$, $p = 0.585$) nor initial plant area ($F_{4,49} = 0.05$, $p = 0.891$) significantly differed across treatments. In a separate model, timing and intensity were tested as interacting explanatory variables with initial plant size added as an additional co-variate. Pollinator metrics, which include total number of pollinator visits, total visitation time per plant, total number of inflorescences visited, visit length per pollinator, and inflorescences visited per pollinator, were analyzed as response variables. I also analyzed total number of inflorescences produced, nectar sugars, and reproductive variables as response variables to the effect of clipping treatments. Reproductive variables tested include total seed number per plant, total seed mass per plant, fruits per inflorescence, individual seed mass, seeds per inflorescence, and seed mass per inflorescence.

I examined all visitation data across hours of the day and saw differences in pollinator visitation between the two periods of observation time: morning/afternoon (7am-1pm) and late-afternoon/evening (1pm-7pm). Since there were three observations per plant, a plant may have two morning/afternoon observations and one afternoon/evening observation, for example. To remove biases due to an unequal distribution of observations between the two time periods, I averaged any duplicate observations in a time period to provide just a single value for the duplicate observation period. When appropriate, data were log-transformed to approximate a normal distribution and my quasi-count data was approximated using a Poisson or negative binomial distribution. Due to a small sample size of Apidae in 2014, and no differences between the two pollinator types in 2015, I combined pollinator groups for both years, except for when analyzing sugars.

Using generalized linear models, I also tested the relationship between pollination and floral traits, and the relationship between pollination and reproduction. Both number of inflorescences and nectar production were analyzed as explanatory variables to pollination metrics. I analyzed nectar sugars using sucrose, glucose and fructose as explanatory variables in the same model to see if nectar composition affected pollinator visitation. In this model, interactions between timing and intensity were non-significant so were removed from the final model. The relationship between reproduction and pollination was examined with fruits per inflorescence, total number of seeds and individual seed mass added as response variables, and pollination metrics as explanatory variables. Pollinator metrics include total number of pollinators that visited, cumulative length of visits per plant, total numbers of flowers visited per plant, visit length per pollinator, and flowers visited per pollinator. Models with count data were tested for significance with a Wald tests, while models with continuous data were assessed using a Type III F-test, both using the car package (Fox and Weisberg 2010). When models were significant, I ran post-hoc comparisons using Tukey tests with the lsmeans package to distinguish which treatments were significantly different from each other (Lenth 2016)

## Results

### *Treatment and floral resources*

Compared to control plants, clipping treatments experienced differences in total inflorescences produced and amount of sugar per flower, but there were no differences in total sugar production or sugar types compared to control plants (Appendix 5). Production of inflorescences was on average lower in clipped compared to control plants in both years, and clipping decreased sugar mass per flower. Timing and intensity of clipping also affected total inflorescences and sugar mass per flower (Table 3.1). Timing of clipping affected total inflorescences produced in 2014, but not in 2015 (Table 3.1). Early clipping produced more inflorescences than late clipping in 2014 (Fig 3.1a). Initial plant size also affected total numbers of flowers produced (Table 3.1). In 2015, on average, each flower produced 0.78 mg of nectar, and the early clipping treatment produced more sugar per flower compared to the late clipping treatment (Table 3.1, Fig 3.2a).

### *Floral resources and pollinator visitation*

In 2014, total inflorescences produced influenced number of pollinator visits, total visit length, total number of flowers visited, but not visit length per pollinator or number of flowers visited per pollinator (Table 3.2). In 2014, as total number of flowers increased, so did number of pollinators (Fig 3.1B, $D^2$=0.23, b=0.00097, SE=0.00028, z= 3.46, p-value<0.001), number of flowers visited (Fig 3.1C, $D^2$= 0.17, b= 0.00095, SE= 0.00029, z= 3.27, p-value<0.001), and time spent pollinating (Fig 3.1D, $D^2$= 0.14, b= 0.00094, SE= 0.00033, z= 2.83, p-value= 0.005).

In 2015, total flowers produced did not affect measures of pollination (Table 3.1). However, flowers with more fructose attracted more pollinators (Table 3.2, Fig 3.3a). Interestingly, more *Bombus* visited when flowers had more fructose ($D^2$= 0.10, b= 11.98, SE= 5.56, z= 2.16, p= 0.031, Fig 3.3b), while Apidae visits declined with increasing sucrose mass in flowers ($D^2$= 0.09, b= -5.61, SE= 2.85, z= -1.967, p= 0.049, Fig 3.3c).

Although total number of pollinator visits was on average lower in clipped compared to control plants, clipped plants did not differ from control plants in terms of total number of flowers visited, total visit length, flowers visited per pollinator or visit length per pollinator

(Appendix 6). Surprisingly, timing and intensity of clipping did not affect total number of pollinator visits (Table 3.3).

### *Treatment effects and pollinator visitation on reproduction*

I did not detect an effect of pollination on most measures of plant reproduction (individual seed mass, number of seeds per inflorescence, and seed mass per inflorescence) in either year (Table 3.4). However, in 2014, as total number of pollinator visits increased, so did number of fruits per inflorescence ($D^2 = 0.13$, b=2.74, SE=1.00, t=2.73, p=0.009, Fig 3.4).

Compared to control plants, clipping affected reproductive measures (Appendix 7); clipped plants on average had decreased fruits per inflorescence, total number of seeds, total mass of seeds and seed mass per inflorescence, while number of seeds per inflorescence, and individual seed mass were unaffected. While intensity of clipping had no effect on reproduction, timing of clipping affected fruits per inflorescence, total number of seeds, total mass of seeds, and seed mass per inflorescence in 2014 (Table 3.5). Number of fruits per inflorescence, number of seeds per plant, mass of seeds per plant, and seed mass per inflorescence were all fewer under late clipping compared to early clipping treatments in 2014 (Table 3.5, Fig 3.5).

## **Discussion**

The magnitude and timing of herbivory can affect plant allocation of resources towards floral components, and this can lead to changes in plant-pollinator interactions, which can subsequently affect plant reproduction. I found that timing and intensity of clipping affected floral components, but visitation rate to the plant was not affected. Although pollinator visitation did not differ across clipping treatments, plant reproduction was affected by treatments. Contrary to my predictions, I found that defoliation later in the season led to decreased floral resources for pollinators and decreased reproduction in cicer milkvetch, and that clipping intensity did not affect plant reproduction. Later clipping negatively affected plant reproduction, but not indirectly through changes in visitation. Although timing and intensity of clipping affected floral resources and seed production, there were minimal effects on pollinator visitation.

### *Intensity of clipping*

Reproductive output of cicer milkvetch did not depend on the magnitude of defoliation. Similarly, in the perennial *Rubus chamaemorus*, neither fruit set, seed set, nor total seed mass per fruit were affected by levels of defoliation ranging from 0-100% (Agren 1989). Intensity of defoliation didn’t affect nectar production or composition, suggesting that moderate levels of grazing don’t limit nectar rewards; however, interactions between intensity and timing of clipping can affect inflorescence numbers, influencing floral resource availability for pollinators. Intensity of defoliation also did not affect nectar production in the perennial *Alstroemeria aurea*, but did affect size of flowers and pollen grains (Aizen and Raffaele 1996). Other studies have found that higher intensities of herbivory negatively affected floral traits and pollinator visitation (Krupnick et al. 1999; Mothershead and Marquis 2000).

### *Timing of clipping and floral components*

When herbivory changes numbers of flowers produced (Strauss 1997; Suárez et al. 2009; Benzel et al. 2009), there can be negative effects on pollination (Klinkhamer et al. 1989; Eckhart 1991; Ohashi and Yahara 1998). I found that, in 2014, plants clipped earlier had higher numbers of inflorescences compared to plants clipped later, and more inflorescences led to increased pollination. The negative impact of late clipping may in part be due lower plasticity for meristems to develop new flower carrying nodes closer to flowering time, as seen in the field gentian, *Gentianella campestris* (Lennartsson et al. 1998). Earlier clipping treatments produced more buds and flower heads in spotted knapweed plants, but pollinator visitation was not tested (Benzel et al. 2009). Early clipping treatments had diminished effects on pollinator visitation of wild radish, *Raphanus raphanistrum*, several weeks after flowering (Lehtilä and Strauss 1997).

Timing of clipping affected floral nectar rewards though changes in total sugar per flower, but did not affect sugar types. Interestingly, visitation by *Bombus* and Apidae increased with increasing fructose levels and decreasing sucrose levels, respectively (Fig. 3.3). Other studies have shown that bumble bees and honey bees prefer higher ratios of sucrose compared to both glucose and fructose (Wykes 1952; Waller 1972; Roldan-Serrano and Guerra-Sanz 2005), but why pollinators prefer certain sugar compositions is still speculative (Wykes 1952; Roldan-Serrano and Guerra-Sanz 2005).

### *Timing of clipping and plant reproduction*

Late clipping during floral investment can have negative consequences for fruit and seed production (Thomson et al. 2002; Anderson and Frank 2003). I found that late clipping in 2014 led to decreased reproductive output. Late defoliation in fireweed, *Epilobium angustifolium,* resulted in reduced weight of both flowers and fruits, as well as lower biomass allocation towards flowers and fruits (Michaud 1991). In part, negative effects on plant reproduction may be due to changes in pollinator visitation following herbivory, but since timing of clipping had a minimal effect on pollinator visitation, it is unlikely changes in visitation would have led to the observed effects of clipping time on reproduction. In *Platanthera bifolia,* hand pollination increased seed set and defoliation decreased seed set, but defoliation did not lead to increased pollen limitation, suggesting that defoliation did not affect plant reproductive success though decreased pollinator attraction (Vallius and Salonen 2006). Insect herbivory on *Rudbeckia hirta* reduced numbers of flowers and pollination rate, but reductions in seed production was mainly due to reduction in flower numbers rather than decreased pollination rates (Hambäck 2001). Similarly, *Isomeris arborea* plants that were exposed to florivory suffered decreased reproduction despite not being pollen limited (Krupnick and Weis 1999). While timing and intensity of clipping did not affect total number of pollinator visits, total number of pollinator visits affected numbers of fruits per inflorescence in 2014. It is possible that observation was insufficient for detecting differences in visitation between treatments in 2014 due to the low number of pollinators in this grassland system. Unfortunately, the different methodology used for observations in each year make it difficult to compare pollinator responses to treatments between years.

### *Annual differences and abiotic factors*

Plants can suffer additive damage from a consecutive year of herbivory (Gustafsson 2004), which can compound effects on floral components and reproduction over time. However, I observed that clipping treatments had a greater effect on the reproduction in the first year rather than in the second year. In part, this could be due to differences in resource allocation between two years or environmental variation. In the second summer, number of inflorescences was affected by initial plant size, but not by clipping treatment. In addition, to timing and intensity of herbivory, plant size at the time of herbivory may also affect production of flowers and fruits.

### *Implications*

My study does not show that grazers indirectly affect pollinator visitation via herbivory. Through the manipulation of the timing and magnitude of herbivory, I found that clipping treatments affected availability of floral resources; however, this did not reduce pollinator visitation to cicer milkvetch. Timing of grazing can affect the reproduction of cicer milkvetch, but cicer milkvetch also demonstrates resiliency with minimal impact to reproduction after two years of clipping. I found that intensity of grazing did not affect floral resources or plant reproduction as much as timing of grazing. By grazing earlier or later in flower development of cicer milkvetch, it may be possible to manage availability of floral resources for pollinators.

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## Tables

Table 3.1. ANOVA results for treatment effect on floral components. Chi-square test results showing treatment variables from a generalized linear model that significantly affected floral traits of cicer milkvetch. I manipulated timing and intensity of clipping, following the same plants over two summers. Degrees of freedom for each variable or group in the model is equal to one.

| | | | timing | | intensity | | initial plant size | | timing x intensity | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Floral traits** | df residual | F-value | **p-value** | F-value | **p-value** | F-value | **p-value** | F-value | **p-value** |
| **2014** | total inflorescences | 43 | 20.94 | **<0.001** | 0.06 | 0.804 | 13.70 | **0.001** | 4.70 | **0.036** |
| **2015** | total inflorescences | 39 | 0.65 | 0.424 | 0.02 | 0.878 | 7.46 | **0.009** | 0.67 | 0.419 |
| | total sugar mass | 33 | 0.29 | 0.594 | 0.92 | 0.344 | 0.35 | 0.558 | NA | NA |
| | sugar mass per flower | 41 | 4.71 | **0.036** | 1.08 | 0.306 | 12.99 | **0.001** | NA | NA |
| | glucose mass per flower | 33 | 5.47 | **0.025** | 0.55 | 0.463 | 0.11 | 0.741 | NA | NA |
| | fructose mass per flower | 33 | 2.38 | 0.132 | 0.21 | 0.652 | 0.41 | 0.524 | NA | NA |
| | sucrose mass per flower | 33 | 3.56 | 0.068 | 0.03 | 0.872 | 0.47 | 0.499 | NA | NA |

Table 3.2. ANOVA results for relationship between floral components and pollination. Chi-square test results showing whether pollination measures were significantly affected by floral components of cicer milkvetch. Variables were fit with generalized linear model. Degrees of freedom for each variable or group in the model is equal to one.

| | | total # of visits | | total flowers visited | | total visit length | | visit length per visitor | | flowers visited per visitor | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Pollination and Floral Triats** | $X^2$ value | p-value | $X^2$ value | p-value | $X^2$ value | p-value | $X^2$ value | p-value | $X^2$ value | p-value |
| **2014** | total infloresences | 11.96 | **0.001** | 10.69 | **0.001** | 8.01 | **0.005** | 0.07 | 0.794 | 0.06 | 0.809 |
| **2015** | total infloresences | 0.69 | 0.407 | 0.23 | 0.633 | 0.27 | 0.603 | 0.02 | 0.897 | 0.08 | 0.776 |
| | total sugar mass | 1.30 | 0.254 | 0.41 | 0.524 | 0.49 | 0.484 | 0.04 | 0.837 | 1.01 | 0.316 |
| | sugar mass per flower | 0.36 | 0.547 | 0.03 | 0.853 | 0.01 | 0.904 | <0.01 | 0.971 | <0.01 | 0.993 |
| | glucose mass per flower | 0.04 | 0.844 | 0.03 | 0.866 | 0.04 | 0.845 | 0.22 | 0.636 | 0.05 | 0.831 |
| | fructose mass per flower | 4.07 | **0.044** | 2.24 | 0.135 | 1.66 | 0.198 | 0.22 | 0.639 | 0.43 | 0.510 |
| | sucrose mass per flower | 0.68 | 0.410 | 0.99 | 0.321 | 0.43 | 0.514 | 0.05 | 0.827 | 0.02 | 0.890 |

Table 3.3. ANOVA results for treatment effect on pollinator visitation. ANOVA F-test results showing treatment variables from a generalized linear model that significantly affected pollinator visitation of cicer milkvetch. Degrees of freedom for each variable or group in the model is equal to one.

| | | timing | intensity | initial plant size | timing x intensity | timing | intensity | initial plant size | timing x intensity |
|---|---|---|---|---|---|---|---|---|---|
| | **Pollination** | $X^2$ value | $X^2$ value | $X^2$ value | $X^2$ value | **p-value** | **p-value** | **p-value** | **p-value** |
| **2014** | total # of visits | 0.29 | 0.01 | 6.71 | 3.21 | 0.587 | 0.928 | **0.010** | 0.073 |
| | total flowers visited | 2.54 | 0.29 | 10.52 | 2.08 | 0.111 | 0.588 | **0.001** | 0.149 |
| | total visit length | 4.51 | 4.47 | 11.16 | 5.03 | **0.034** | **0.035** | **0.001** | **0.025** |
| | visit length per visitor | 2.96 | 0.44 | 1.87 | 2.54 | 0.085 | 0.506 | 0.172 | 0.111 |
| | flowers visited per visitor | 1.16 | 0.57 | 0.64 | 0.35 | 0.281 | 0.450 | 0.425 | 0.553 |
| **2015** | total # of visits | 0.05 | 0.17 | 0.19 | 0.02 | 0.816 | 0.677 | 0.659 | 0.896 |
| | total flowers visited | 0.00 | 0.45 | 1.14 | 0.00 | 0.997 | 0.504 | 0.285 | 0.980 |
| | total visit length | 0.07 | 0.92 | 1.33 | 0.02 | 0.792 | 0.337 | 0.249 | 0.885 |
| | visit length per visitor | 0.73 | 0.54 | 0.79 | 0.03 | 0.392 | 0.461 | 0.376 | 0.862 |
| | flowers visited per visitor | 0.44 | 0.18 | 0.28 | 0.00 | 0.505 | 0.668 | 0.598 | 0.988 |

Table 3.4. ANOVA results for relationship between pollination and reproduction. Chi-square test results showing whether pollination significantly affected reproduction of cicer milkvetch. Variables were fit with generalized linear model. Degrees of freedom for each variable or group in the model is equal to one.

| | | total # of visits | | | total flowers visited | | | total visit length | | | visit length per visitor | | | flowers visited per visitor | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Pollination and Reproduction** | F-value | df residual | p-value | F-value | df residual | p-value | F-value | df residual | p-value | F-value | df residual | p-value | F-value | df residual | p-value |
| **2014** | fruits per inflorescence | 7.44 | 48 | **0.009** | 2.25 | 43 | 0.141 | 2.98 | 42 | 0.092 | 1.94 | 17 | 0.181 | 0.51 | 19 | 0.484 |
| | individual seed mass | 1.90 | 48 | 0.175 | 0.26 | 43 | 0.614 | 1.73 | 42 | 0.196 | 1.09 | 17 | 0.311 | 0.79 | 19 | 0.384 |
| | # seeds per inflorescence | 3.61 | 48 | 0.063 | 1.91 | 46 | 0.174 | 1.13 | 48 | 0.294 | 0.54 | 17 | 0.471 | 1.16 | 19 | 0.294 |
| | seed mass per inflorescence | 2.93 | 43 | 0.093 | 2.23 | 43 | 0.143 | 1.82 | 42 | 0.185 | 0.83 | 17 | 0.375 | 0.57 | 19 | 0.460 |
| **2015** | fruits per inflorescence | 1.52 | 53 | 0.224 | 1.46 | 53 | 0.233 | 2.92 | 53 | 0.093 | 0.81 | 39 | 0.374 | 0.57 | 39 | 0.453 |
| | individual seed mass | 0.01 | 53 | 0.914 | 0.20 | 53 | 0.660 | 1.05 | 53 | 0.310 | 1.29 | 39 | 0.262 | 0.04 | 39 | 0.846 |
| | # seeds per inflorescence | 0.21 | 53 | 0.652 | 0.72 | 53 | 0.399 | 0.72 | 53 | 0.399 | 3.25 | 39 | 0.079 | 2.81 | 39 | 0.102 |
| | seed mass per inflorescence | 0.14 | 53 | 0.710 | 0.72 | 53 | 0.399 | 0.72 | 53 | 0.399 | 1.76 | 39 | 0.193 | 2.19 | 39 | 0.147 |

Table 3.5. ANOVA results for treatment effect on plant reproduction. ANOVA F-test results showing treatment variables from a generalized linear model that significantly affected reproduction of cicer milkvetch. Degrees of freedom for each variable or group in the model is equal to one.

| | | timing | intensity | initial plant size | timing x intensity | timing | intensity | initial plant size | timing x intensity |
|---|---|---|---|---|---|---|---|---|---|
| **reproduction** | df residual | F-value | F-value | F-value | F-value | **p-value** | **p-value** | **p-value** | **p-value** |
| fruits per inflorescence | 43 | 6.28 | 0.02 | 1.27 | 0.26 | **0.016** | 0.891 | 0.265 | 0.611 |
| total number of seeds | 43 | 23.53 | 0.10 | 14.49 | 3.01 | **<0.001** | 0.759 | **<0.001** | 0.090 |
| total mass of seeds | 43 | 25.57 | 0.13 | 14.35 | 3.37 | **<0.001** | 0.721 | **<0.001** | 0.074 |
| individual seed mass | 43 | 13.26 | 0.47 | 1.79 | 1.99 | **0.001** | 0.495 | 0.187 | 0.166 |
| # seeds per inflorescence | 43 | 1.97 | 0.04 | 0.06 | 0.12 | 0.167 | 0.837 | 0.808 | 0.730 |
| seed mass per inflorescence | 43 | 5.09 | 0.33 | 0.40 | 0.74 | **0.029** | 0.569 | 0.533 | 0.396 |
| fruits per inflorescence | 40 | 1.39 | 0.66 | 0.00 | 0.22 | 0.246 | 0.423 | 0.948 | 0.641 |
| total number of seeds | 39 | 0.17 | 0.00 | 2.56 | 0.50 | 0.685 | 0.972 | 0.118 | 0.484 |
| total mass of seeds | 39 | 0.32 | 0.01 | 2.67 | 0.38 | 0.573 | 0.931 | 0.110 | 0.539 |
| individual seed mass | 39 | 4.84 | 3.42 | 0.22 | 3.52 | **0.034** | 0.072 | 0.644 | 0.068 |
| # seeds per inflorescence | 39 | 0.18 | 0.25 | 0.87 | 0.56 | 0.678 | 0.619 | 0.357 | 0.460 |
| seed mass per inflorescence | 39 | 0.10 | 1.19 | 0.55 | 1.46 | 0.755 | 0.282 | 0.462 | 0.235 |

## Figures

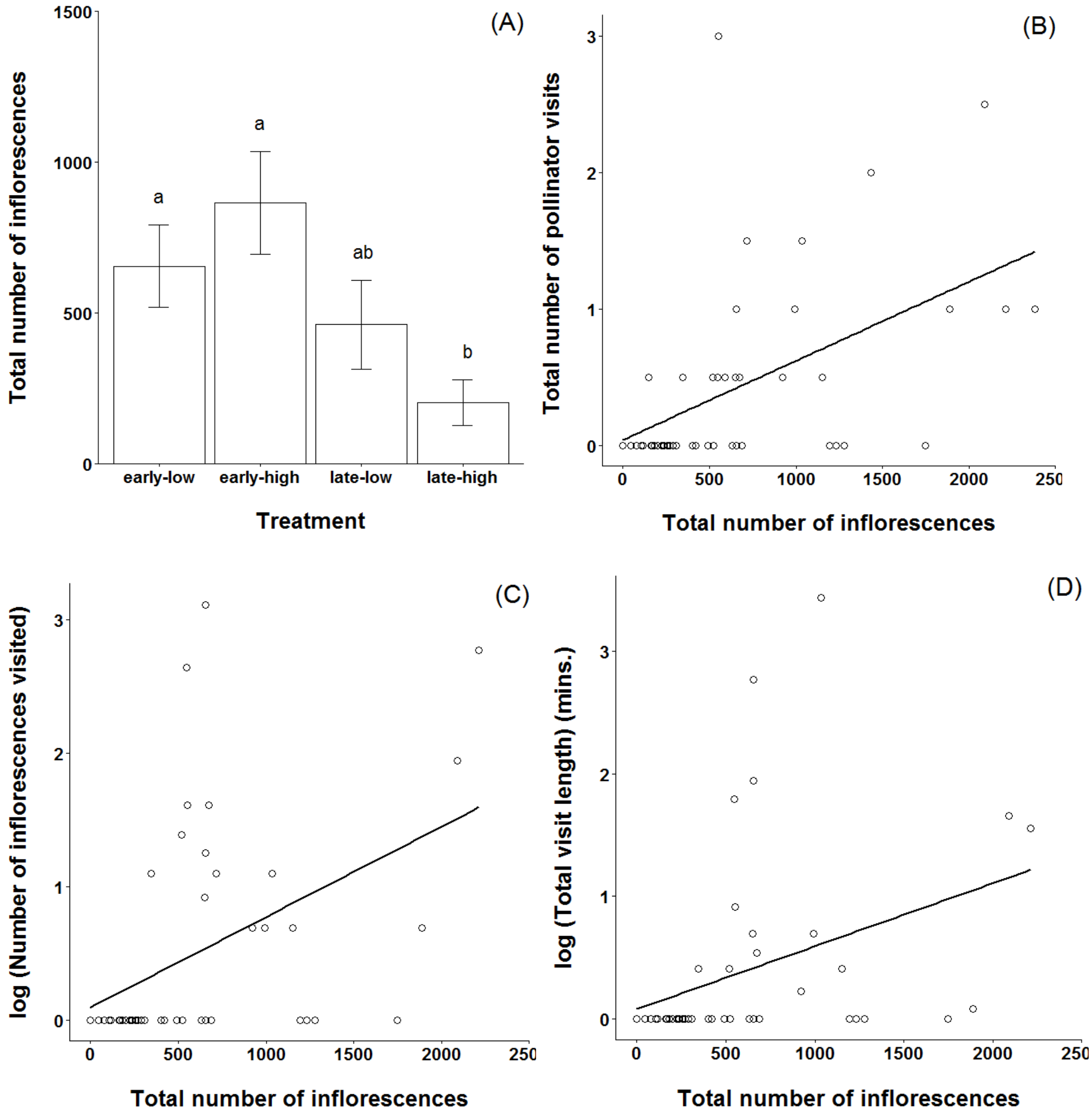

Figure 3.1. Treatment effect on number of inflorescences and relationship between inflorescence numbers to pollinator visitation. A) Mean (+/- SE) of pollinator visitation across clipping treatments where values are relative to control plants. Lower case letters represent significant differences between groups (Tukey test, P<0.05). Graphs represents relationships between the number of inflorescences and B) total pollinator visits, C) total inflorescences visited, D) visit length per pollinator. Pollinator counts include averaged values. Fitted lines are all linear regressions.

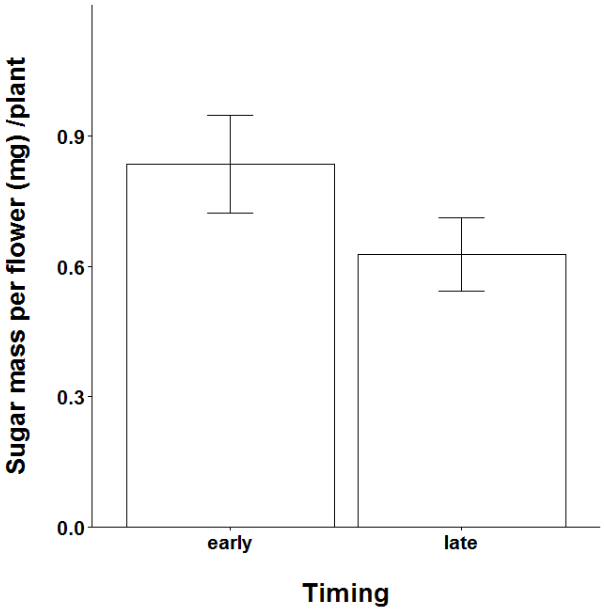

Figure 3.2. Mean (+/- SE) of sugar mass per flower measured in 2015. Nectar was collected from 25 flowers per plant and analyzed for three dominant sugar types: sucrose, glucose and fructose.

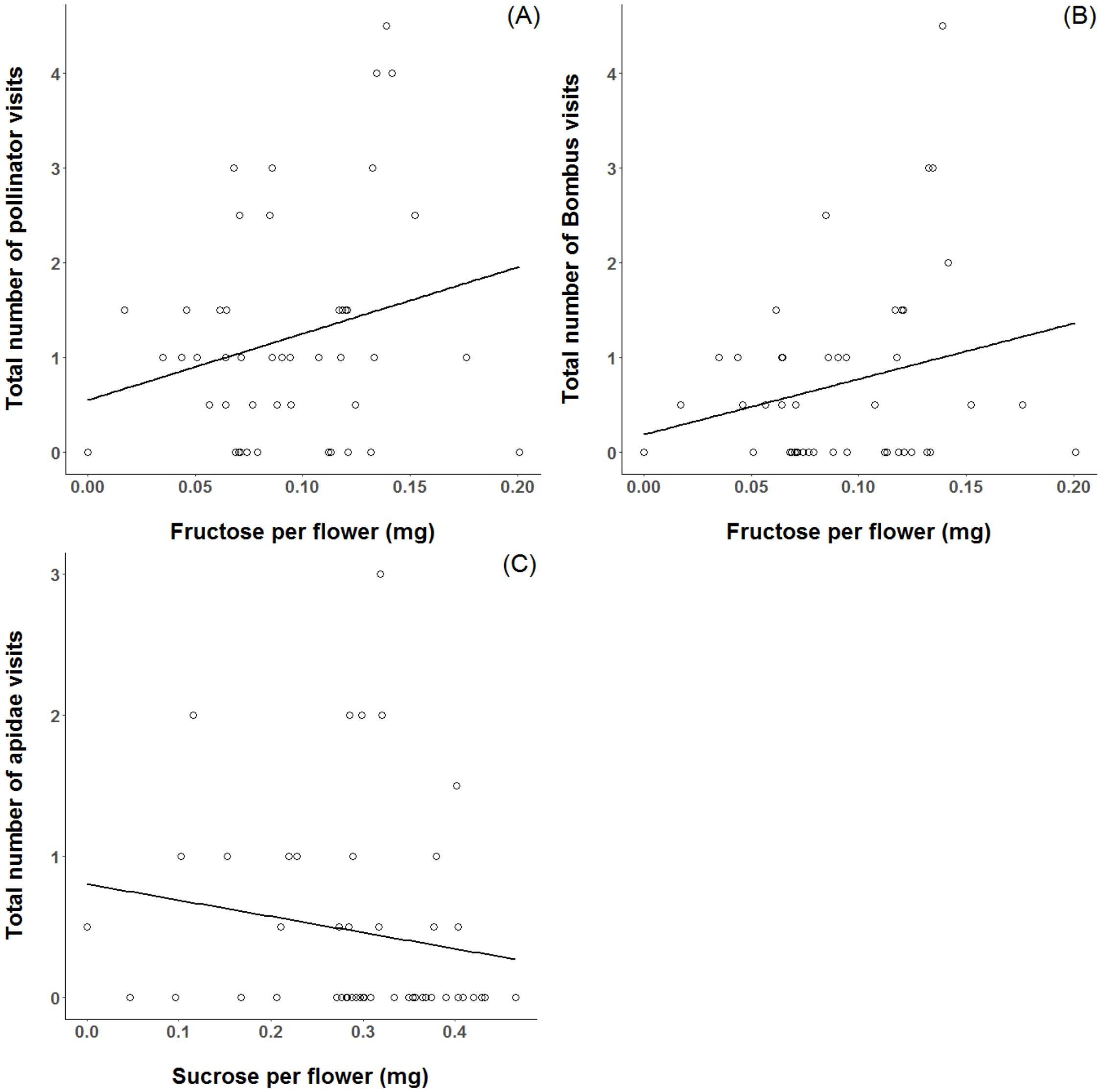

Figure 3.3. Relationship between pollinator visitation and nectar sugars. All graphs are fitted with a linear regression. A) Total pollinators visited across fructose amounts per flower, B) Total number of *Bombus* visits across fructose amounts per flower, C) Total number of apidae visits across amounts of sucrose per flower. Pollinator counts include averaged values. Fitted lines are all linear regressions.

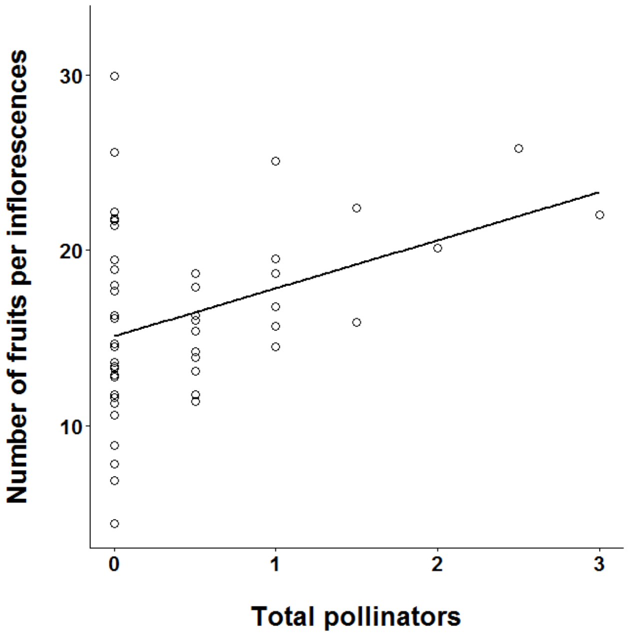

Figure 3.4. Relationship between pollinator visitation and reproduction in 2014. Total pollinators regressed against number of fruits per inflorescence. Pollinator counts include averaged values and graph is fitted with a linear regression.

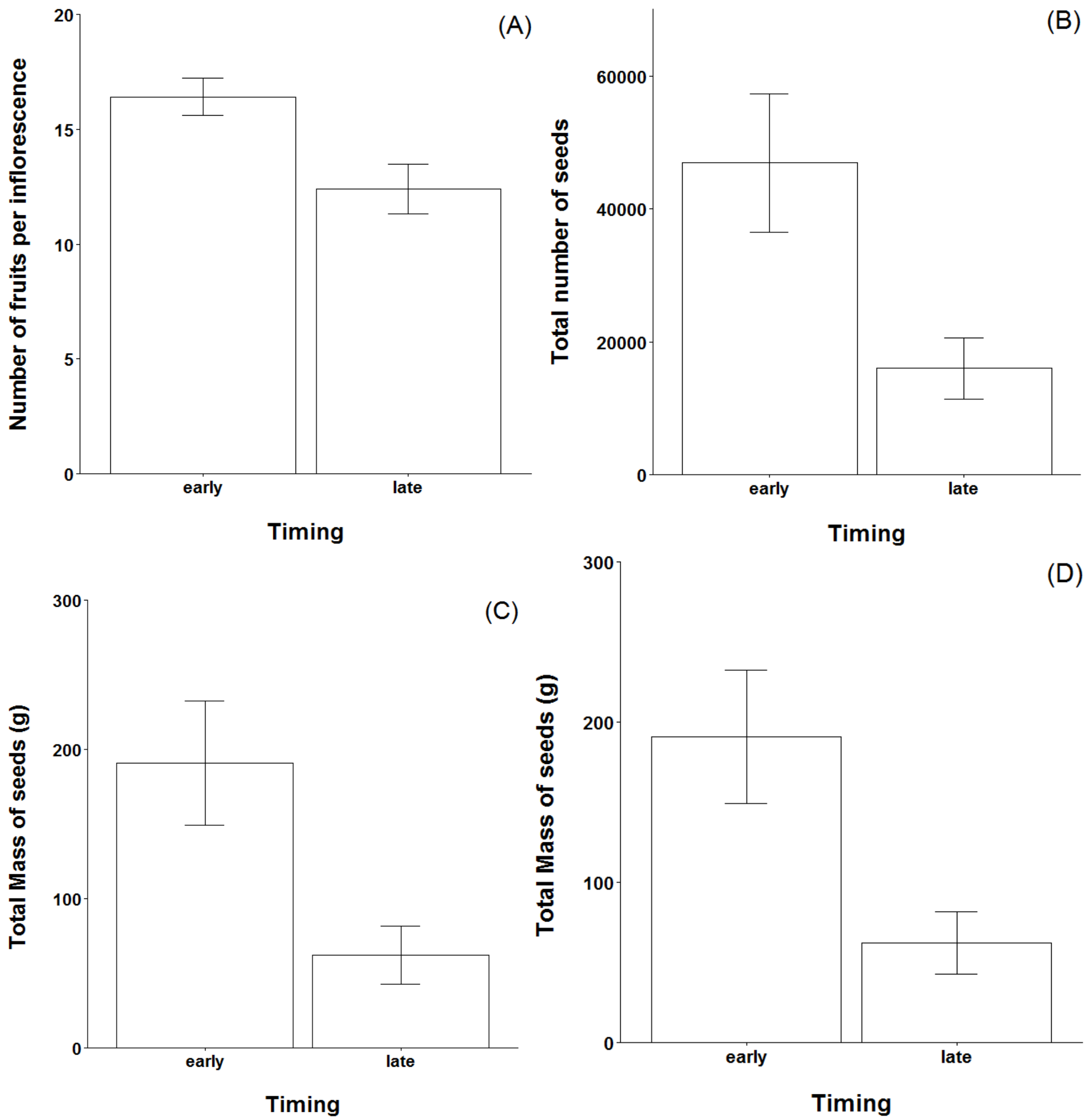

Figure 3.5. Measures of reproduction across clipping times for 2014. Mean (+/- SE) where all values are relative to control plants. A) Fruits per inflorescence, B) Total number of seeds, C) Total mass of seeds, D) Seed mass per inflorescence

## Chapter 4: Concluding Remarks

Herbivory can have significant consequences for plant growth and reproduction, directly through limitations in resources and indirectly through effects on pollinators (Mothershead and Marquis 2000; Gómez and Olivieri 2003). Responses to herbivory such as increased growth and decreased root stores are a common consequence, (McNaughton 1983; Belsky et al. 1993; Strauss and Agrawal 1999; Kessler and Baldwin 2002). Herbivory can also affect interactions between plants and pollinators by altering floral traits which can have consequences for reproduction (Quesada et al. 1995; Suárez et al. 2009; Conner and Rush 1996). This thesis adds to a growing body of research concerning herbivore-plant-pollinator interactions. The goal of this thesis was to, first, understand how grazing affects the growth and reproduction of an important forage crop, and, second, to explore whether grazing affects plant preference by pollinators, thereby affecting subsequent pollination and reproduction.

In Chapter one, I have shown that cicer milkvetch (*Astraglus cicer*), a legume introduced from Europe, can produce similar amounts of vegetative growth regardless of timing or intensity of clipping. However, timing and intensity of clipping affected plant reproduction, with plants in the late-high intensity treatment experiencing the highest reductions in reproduction. Reproductive biomass was generally lower compared to vegetative biomass when plants were clipped later in the season; however, this effect was present only in the first year. Previous studies have demonstrated either positive, neutral or negative plant responses to herbivory (McNaughton 1979; Mauricio et al. 1993; Lennartsson et al. 1998; Hickman and Hartnett 2002; García and Ehrlén 2002; Striker et al. 2011). My results show that, in part, negative responses in plant reproduction may be mediated by timing of herbivory.

For perennial plants, herbivory can occur for multiple growing seasons that can lead to additive negative effects (Gustafsson 2004). Surprisingly, rather than compounding effects, a second year of clipping led to no differences in growth and reproduction between treatments, demonstrating cicer's high tolerance to grazing. Few studies have simultaneously examined effects of timing and intensity on perennial plant growth and reproduction, and I have demonstrated that both factors affect plant reproduction. The study response to herbivory in agronomic crops can have direct agricultural applications, but also furthers our understanding of how escaped agronomics such as sweet clover and alfalfa may persist in the environment.

In Chapter two, I found that interactions between timing and intensity of clipping affected the number of inflorescences produced, while only timing of clipping affected nectar production and measures of plant reproduction. However, treatments did not affect other measures of pollinator visitation. Aside from effects on nectar production, effects on number of inflorescences, pollinator visitation and reproduction were mostly limited to the first year of study. Although more inflorescences led to increased pollinator visits in 2014, numbers of flowers visited and visit length, pollinator visitation had a surprisingly weak effect on plant reproduction. Therefore, herbivory may not affect plant reproduction indirectly though pollinators, but may have a direct effect by affecting plant resource availability. Other studies involving relationships between herbivory, pollination and reproduction also demonstrate a complex story; results can be divided into two scenarios, one in which herbivory negatively affects reproduction with no effects on pollination (Krupnick and Weis 1999; Hambäck 2001; Vallius and Salonen 2006; Narbona and Dirzo 2010), and the other, where herbivory negatively affects pollination and subsequently reduces reproduction (Strauss et al. 1996; Lehtilä and

Strauss 1997; Mothershead and Marquis 2000). Our results align with the first scenario, where effects on reproduction are due to resource limitation following herbivory. Limited resources can lead to trade-offs between growth and reproduction, as seen in chapter 1, where plants invested fewer resources into fruit biomass relative to vegetative biomass when clipped later in the season. This aligns with results from chapter 2, where a later clipping also negatively affected total flower production, seed number and seed size.

Interestingly, despite many studies that show increasing pollinator visitation with increasing flower number (Klinkhamer and de Jong 1990; Strauss et al. 1996; Ohashi and Yahara 1998), no relationship was observed between numbers of inflorescences and numbers of pollinators in 2015. This may suggest that pollinators did not discriminate between plants based on flower numbers and this is supported by the observation of fewer co-flowering species in the field, which may increase competition for limited flowering resources (Morales and Traveset 2008; Mitchell et al. 2009). Many studies have examined the effects of intensity of herbivory on floral allocation, pollination and plant reproduction (Krupnick et al. 1999; Mothershead and Marquis 2000; Narbona and Dirzo 2010), but there are no studies, to my knowledge, that have explored the effects of timing of herbivory on all three components in tandem. Since I only examined effects on female fitness of plants, future studies on the impact of timing and intensity of defoliation on male fitness will be important for understanding how interactions between herbivory and pollination affect total plant fitness.

Cicer milkvetch is a valuable forage crop for cattle, benefits the soil by fixing nitrogen, and provides a large source of floral resources for pollinators (Richards 1987; Kephart et al.

1990). Thus, maintaining cicer milkvetch in rangelands may be desirable for rangeland management. My results indicate that cicer can sustain low to moderate levels of grazing with minimal impact on plant growth and reproduction after two years. However, grazing during a late stage in flower bud formation can negatively affect both floral resources available for pollinators and plant reproduction. These results may be generalizable to other flowering legumes, but more so for agronomic legumes such as alfalfa and sweet clover, which possess traits that enable them to tolerate grazing.

Cicer milkvetch is also a non-native legume spreading naturally into native grasslands, which may compete with other native plants, not only for abiotic resources, but also for pollinators. As such, understanding cicer milkvetch's response to grazing and how grazing affects its interactions with native pollinators may be important for conserving grassland biodiversity. Future studies on pollen limitation in cicer milkvetch, heterospecific pollen transfer to other flowering legumes, and density-dependent effects on pollination of native plants will help in understanding its relationship with native plants and pollinators.

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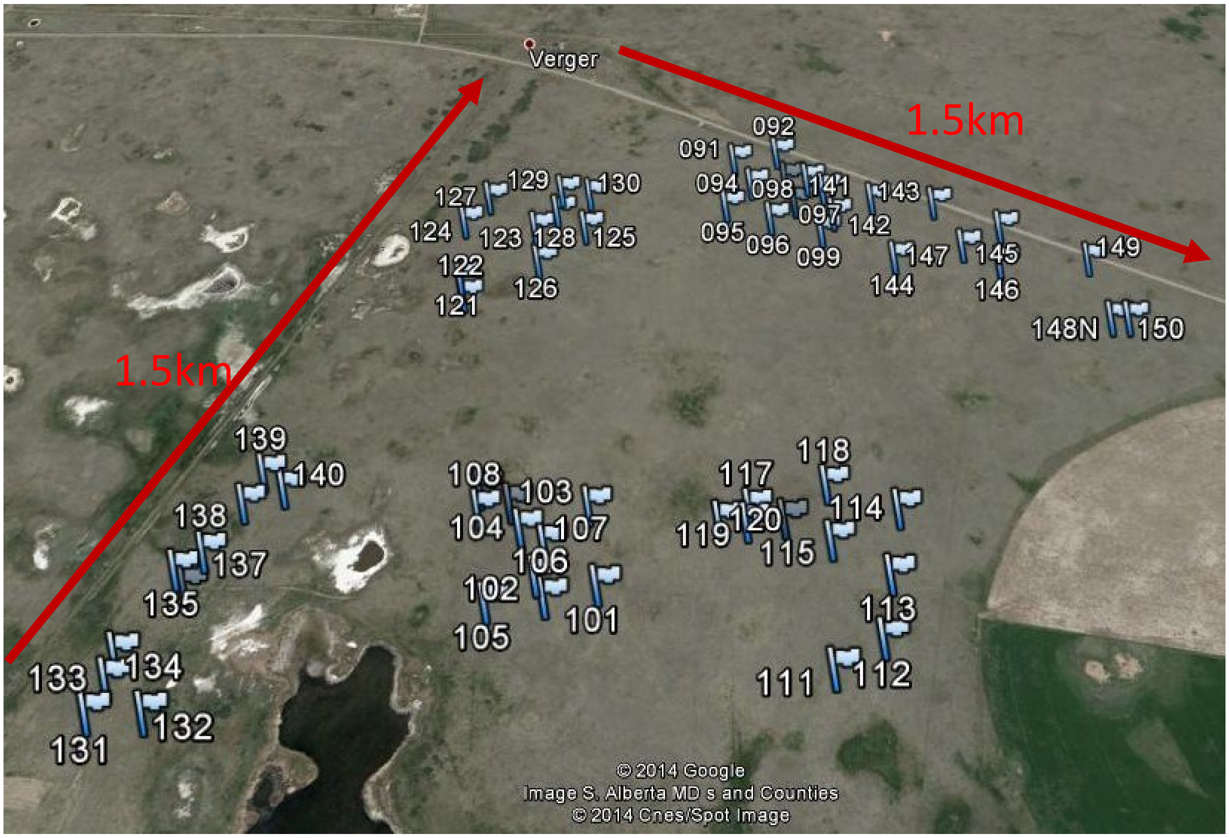
# Appendices

Appendix 1. Map of plant locations in field site

At Mattheis ranch, GPS co-ordinates were taken at each experimental plant in the field.



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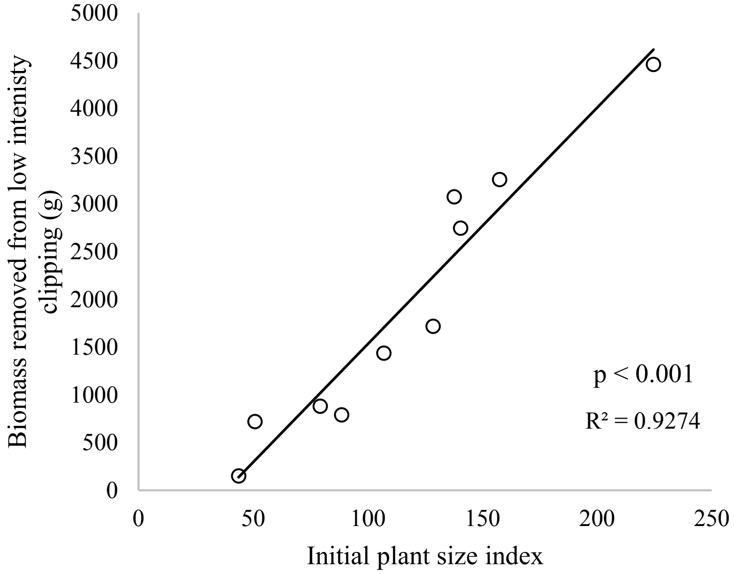
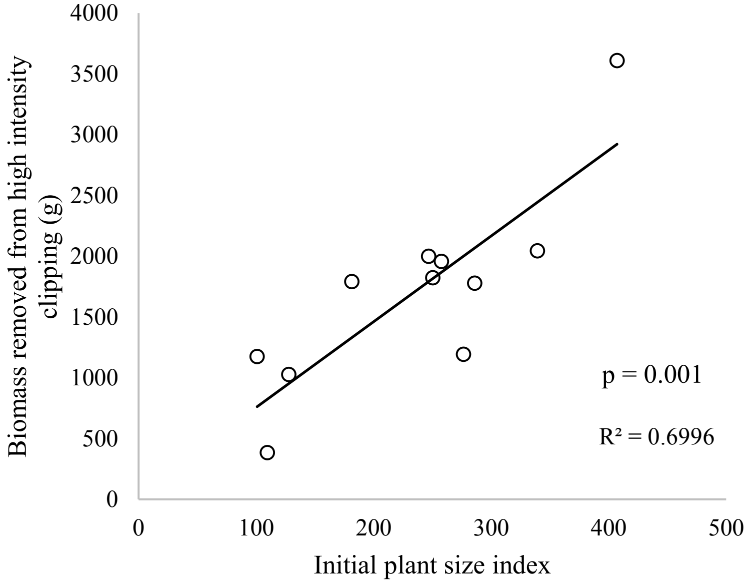
## Appendix 2. Regression of plant biomass VS initial plant size

Prior to early clipping, I measured initial stem length and counted total number of stems for all plants, including control and late clipping treatments. Initial plant size index is calculated from total number of plant stems multiplied by average initial stem length. Plant biomass removed from early clipping treatments was dried and weighed. The top graph shows biomass (g) removed from early-low intensity clipping, while the bottom graph is from early-high intensity clipping, and both are regressed across an index of initial plant size.

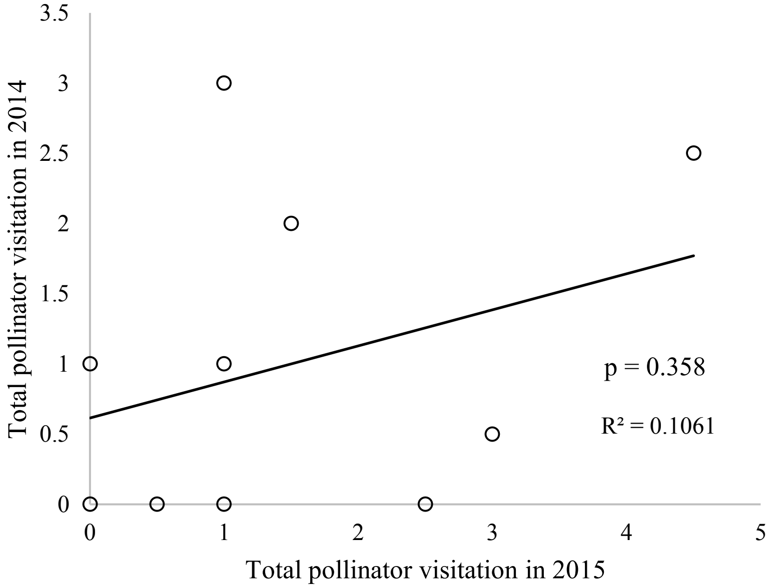




## Appendix 3. Camera observations VS in person observations

Some studies have used both in person observation and camera observations interchangeably (Johnson et al. 2010, Georgian et al. 2015). Further, it is possible to accurately capture pollinator abundance using video cameras. In a study by Zych (2002), there was a high correlation between numbers of pollinators observed in camera observations and numbers of pollinators captured 20 minutes following observations. However, no studies that I am aware of have looked at differences between in person observations and camera observations.

I observed pollinator visitation to plants in person in 2014 and did camera observations in 2015. Since camera observations have not been verified to see if they correlated with in person observation, I avoided any direct comparisons between the two years of pollination data. A regression of total pollinator visits to control plants in 2014 and 2015 was also not strongly correlated.



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## Appendix 4. Chapter 2 data: differences to control plants

Table shows significant differences between control plants and each clipping treatment in a Dunnett's test, resulting in four comparisons tested.

| | **Early-high** | | | | **Early-low** | | | | **Late-high** | | | | **Late-low** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Growth** | Estimate | Standard Error | z-value | **p-value** | Estimate | Standard Error | z-value | **p-value** | Estimate | Standard Error | z-value | **p-value** | Estimate | Standard Error | z-value | **p-value** |
| Change in stem count | 0.51 | 23.33 | 0.02 | 1.000 | 18.86 | 23.80 | 0.79 | 0.846 | 35.18 | 22.73 | 1.55 | 0.342 | -11.24 | 23.99 | -0.47 | 0.973 |
| Change in spread ($m^2$) | -0.71 | 0.27 | -2.65 | **0.028** | -0.25 | 0.27 | -0.93 | 0.764 | -0.71 | 0.26 | -2.74 | **0.022** | -0.77 | 0.27 | -2.81 | **0.018** |
| Shoot growth (cm) | 0.58 | 1.59 | 0.36 | 0.989 | -2.50 | 1.63 | -1.54 | 0.346 | -3.52 | 1.55 | -2.27 | 0.078 | -5.19 | 1.64 | -3.17 | **0.006** |
| Vegetative biomass (g) | -243.60 | 142.80 | -1.71 | 0.259 | -245.50 | 147.10 | -1.67 | 0.278 | -398.00 | 139.10 | -2.86 | **0.015** | -469.30 | 146.80 | -3.20 | **0.006** |
| Leaf biomass(g) | 5.55 | 57.92 | 0.10 | 1.000 | -30.05 | 59.92 | -0.50 | 0.965 | -29.84 | 56.42 | -0.53 | 0.958 | -107.14 | 59.55 | -1.80 | 0.218 |
| Stem biomass (g) | -0.31 | 0.31 | -0.99 | 0.720 | -0.39 | 0.32 | -1.24 | 0.540 | -0.61 | 0.30 | -2.04 | 0.133 | -0.84 | 0.32 | -2.64 | **0.030** |
| leaves:stems | 0.25 | 0.10 | 2.63 | **0.030** | 0.09 | 0.10 | 0.92 | 0.767 | 0.35 | 0.09 | 3.78 | **<0.001** | 0.22 | 0.10 | 2.21 | **0.089** |
| **Reproduction** | | | | | | | | | | | | | | | | |
| Fruit biomass (g) | -0.25 | 0.47 | -0.54 | 0.955 | -0.44 | 0.48 | -0.91 | 0.774 | -2.04 | 0.45 | -4.51 | **<0.001** | -1.53 | 0.48 | -3.22 | **0.005** |
| Fruits:(veg. biomass) | -0.05 | 0.09 | -0.59 | 0.939 | -0.06 | 0.09 | -0.69 | 0.899 | -0.40 | 0.08 | -4.75 | **<0.001** | -0.23 | 0.09 | -2.60 | **0.033** |

Appendix 5. Chapter 3 floral data: differences to control plants

Table shows significant differences between control plants and each clipping treatment in a Dunnett's test, resulting in four comparisons tested.

| | | **Early-high** | | | | **Early-low** | | | | **Late-high** | | | | **Late-low** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Floral traits** | Estimate | Standard Error | z-value | **p-value** | Estimate | Standard Error | z-value | **p-value** | Estimate | Standard Error | z-value | **p-value** | Estimate | Standard Error | z-value | **p-value** |
| **2014** | total inflorescences | 0.00 | 0.01 | -0.05 | 1.000 | 0.00 | 0.01 | 0.27 | 0.997 | 0.07 | 0.02 | 4.74 | **<0.001** | 0.03 | 0.01 | 2.13 | 0.114 |
| **2015** | total inflorescences | -0.42 | 0.46 | -0.92 | 0.769 | -0.35 | 0.46 | -0.75 | 0.871 | -0.82 | 0.44 | -1.86 | 0.192 | -1.28 | 0.44 | -2.91 | **0.013** |
| | total sugar mass | -0.77 | 0.80 | -0.97 | 0.732 | -1.74 | 0.75 | -2.32 | 0.068 | -1.51 | 0.76 | -2.00 | 0.143 | -1.69 | 0.77 | -2.20 | 0.090 |
| | sugar mass per flower | -0.27 | 0.19 | -1.44 | 0.401 | -0.06 | 0.18 | -0.33 | 0.992 | -0.46 | 0.18 | -2.56 | **0.037** | -0.40 | 0.18 | -2.26 | 0.078 |
| | glucose mass per flower | -0.01 | 0.01 | -0.46 | 0.975 | 0.00 | 0.01 | -0.18 | 0.999 | -0.03 | 0.01 | -2.19 | 0.094 | -0.02 | 0.01 | -1.51 | 0.367 |
| | fructose mass per flower | -0.01 | 0.02 | -0.32 | 0.993 | -0.01 | 0.02 | -0.73 | 0.883 | -0.03 | 0.02 | -1.36 | 0.464 | -0.03 | 0.02 | -1.60 | 0.319 |
| | sucrose mass per flower | 0.01 | 0.05 | 0.12 | 1.000 | -0.02 | 0.05 | -0.48 | 0.971 | -0.08 | 0.05 | -1.79 | 0.225 | -0.07 | 0.05 | -1.41 | 0.428 |

Appendix 6. Chapter 3 pollination data: differences to control plants

Table shows significant differences between control plants and each clipping treatment in a Dunnett’s test, resulting in four comparisons tested.

| | | **Early-high** | | | | **Early-low** | | | | **Late-high** | | | | **Late-low** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Pollination** | Estimate | Standard Error | z-value | **p-value** | Estimate | Standard Error | z-value | **p-value** | Estimate | Standard Error | z-value | **p-value** | Estimate | Standard Error | z-value | **p-value** |
| **2014** | total # of visits | -0.69 | 0.54 | -1.28 | 0.577 | -2.01 | 0.88 | -2.29 | 0.083 | -2.25 | 0.90 | -2.51 | **0.047** | -1.11 | 0.67 | -1.65 | 0.326 |
| | total flowers visited | -0.02 | 0.59 | -0.03 | 1.000 | -0.37 | 0.62 | -0.59 | 0.946 | -1.51 | 0.96 | -1.58 | 0.338 | -0.19 | 0.63 | -0.30 | 0.996 |
| | total visit length | 0.91 | 0.67 | 1.36 | 0.488 | -0.65 | 0.81 | -0.80 | 0.862 | -2.50 | 1.66 | -1.50 | 0.391 | -0.05 | 0.71 | -0.07 | 1.000 |
| | visit length per visitor | 0.69 | 0.70 | 0.99 | 0.745 | 0.18 | 0.84 | 0.22 | 0.999 | -2.42 | 1.75 | -1.39 | 0.464 | -0.02 | 0.84 | -0.02 | 1.000 |
| | flowers visited per visitor | 0.18 | 0.63 | 0.29 | 0.996 | 0.67 | 0.65 | 1.02 | 0.699 | -0.91 | 1.03 | -0.88 | 0.792 | 0.22 | 0.75 | 0.30 | 0.995 |
| **2015** | total # of visits | -0.35 | 0.40 | -0.89 | 0.804 | -0.18 | 0.38 | -0.48 | 0.973 | -0.47 | 0.40 | -1.18 | 0.603 | -0.22 | 0.38 | -0.57 | 0.951 |
| | total flowers visited | -0.61 | 0.34 | -1.79 | 0.235 | -0.37 | 0.32 | -1.17 | 0.624 | -0.66 | 0.34 | -1.96 | 0.167 | -0.41 | 0.32 | -1.28 | 0.546 |
| | total visit length | -0.93 | 0.52 | -1.76 | 0.253 | -0.39 | 0.44 | -0.88 | 0.822 | -0.84 | 0.50 | -1.69 | 0.289 | -0.38 | 0.44 | -0.87 | 0.831 |
| | visit length per visitor | -0.96 | 0.60 | -1.60 | 0.341 | -0.48 | 0.51 | -0.93 | 0.789 | -0.48 | 0.53 | -0.90 | 0.811 | -0.09 | 0.49 | -0.17 | 1.000 |
| | flowers visited per visitor | -0.62 | 0.37 | -1.67 | 0.298 | -0.45 | 0.35 | -1.28 | 0.543 | -0.39 | 0.35 | -1.11 | 0.667 | -0.18 | 0.35 | -0.51 | 0.970 |

Appendix 7**.** Chapter 3 reproductive data: differences to control plants

Table shows significant differences between control plants and each clipping treatment in a Dunnett's test, resulting in four comparisons tested.

| | | **Early-high** | | | | **Early-low** | | | | **Late-high** | | | | **Late-low** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **reproduction** | Estimate | Standard Error | z-value | **p-value** | Estimate | Standard Error | z-value | **p-value** | Estimate | Standard Error | z-value | **p-value** | Estimate | Standard Error | z-value | **p-value** |
| **2014** | fruits per inflorescence | -4.95 | 1.96 | -2.53 | **0.040** | -4.55 | 1.96 | -2.32 | 0.069 | -9.99 | 1.96 | -5.09 | **<0.001** | -8.13 | 1.96 | -4.15 | **<0.001** |
| | total number of seeds | -20538 | 17655 | -1.16 | 0.594 | -40951 | 17695 | -2.31 | 0.069 | -74887 | 17703 | -4.23 | **<0.001** | -56679 | 17656 | -3.21 | **0.005** |
| | total mass of seeds | -0.17 | 0.54 | -0.31 | 0.994 | -0.38 | 0.55 | -0.69 | 0.898 | -3.06 | 0.55 | -5.60 | **<0.001** | -1.79 | 0.54 | -3.29 | **0.004** |
| | individual seed mass | 0.38 | 0.23 | 1.64 | 0.290 | 0.21 | 0.23 | 0.89 | 0.784 | -0.49 | 0.23 | -2.09 | 0.119 | -0.19 | 0.23 | -0.79 | 0.844 |
| | # seeds per inflorescence | -8.52 | 13.14 | -0.65 | 0.916 | -10.55 | 13.17 | -0.80 | 0.840 | -27.26 | 13.18 | -2.07 | 0.123 | -22.74 | 13.14 | -1.73 | 0.246 |
| | seed mass per inflorescence | -11.73 | 49.02 | -0.24 | 0.998 | -37.89 | 49.13 | -0.77 | 0.857 | -123.52 | 49.15 | -2.51 | **0.041** | -90.25 | 49.02 | -1.84 | 0.199 |
| **2015** | fruits per inflorescence | 1.28 | 1.74 | 0.74 | 0.875 | -0.10 | 1.78 | -0.06 | 1.000 | -0.84 | 1.69 | -0.49 | 0.966 | -1.03 | 1.69 | -0.61 | 0.931 |
| | total number of seeds | -38140 | 20188 | -1.89 | 0.181 | -29494 | 20238 | -1.46 | 0.395 | -32870 | 19229 | -1.71 | 0.256 | -43103 | 19224 | -2.24 | 0.083 |
| | total mass of seeds | -0.34 | 0.78 | -0.44 | 0.978 | -0.41 | 0.78 | -0.53 | 0.958 | -0.84 | 0.74 | -1.13 | 0.618 | -1.62 | 0.74 | -2.18 | 0.097 |
| | individual seed mass | 0.37 | 0.21 | 1.77 | 0.231 | -0.05 | 0.21 | -0.22 | 0.998 | -0.11 | 0.20 | -0.54 | 0.953 | 0.06 | 0.21 | 0.27 | 0.996 |
| | # seeds per inflorescence | 0.74 | 6.81 | 0.11 | 1.000 | -2.76 | 6.99 | -0.40 | 0.985 | 2.91 | 6.64 | 0.44 | 0.978 | 6.99 | 6.77 | 1.03 | 0.689 |
| | seed mass per inflorescence | 19.95 | 27.00 | 0.74 | 0.874 | -10.72 | 27.70 | -0.39 | 0.986 | 8.75 | 26.32 | 0.33 | 0.992 | 26.20 | 26.85 | 0.98 | 0.729 |