

THESIS

PLANT-INSECT INTERACTIONS AND MANAGEMENT OPTIONS FOR
VERBASCUM THAPSUS L. IN COLORADO

Submitted by

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Graduate Degree Program in Ecology

In partial fulfillment of the requirements

For the Degree of Masters of Science

Colorado State University

Fort Collins, Colorado

Fall 2011

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ABSTRACT

PLANT-INSECT INTERACTIONS AND MANAGEMENT OPTIONS FOR *VERBASCUM THAPSUS* L. IN COLORADO

In the field of invasion biology, understanding the ecology of a species goes hand and hand with understanding management options. Common mullein (*Verbascum thapsus* L.) is a non-native plant introduced to North America from Europe in the 1700s. It has naturalized and spread throughout North America, with populations establishing after disturbance. Through a series of field experiments, I tested the effect of insect herbivory on the growth of *V. thapsus* in Colorado and also investigated management options for the reproductive stage of this biennial plant.

Verbascum thapsus, while widespread, is rarely problematic in undisturbed habitats. This may be due to interactions with new community members, such as insect herbivores, that limit population size. Starting in 2009, I followed a population of *V. thapsus* plants in a disturbed landscape in Loveland, Colorado. A subset of the population was protected from herbivores with insecticides, reducing overall herbivore damage during the bolting year, for the first year, and throughout the lifecycle of the plants. Reducing herbivory in the first year increased the size that *V. thapsus* rosettes reached by the fall. Reducing herbivory during only the bolting year of the plant increased the height of the bolting plants. Reducing herbivory over both growing seasons increased plant

height, the length of the reproductive stalk, and seed set. Plants with reduced herbivory also had higher rates of survival. Thus, insect herbivory reduced performance of *V. thapsus* in this study and may be one of the factors that prevents this introduced plant from dominating its recipient communities.

Mechanical control of *V. thapsus* via digging or cutting stalks is considered the best option for management. Current practice encourages the collection of reproductive stalks from the field to limit the spread of seeds. While effective, this substantially increases the labor involved in management. I tested the viability of *V. thapsus* seeds collected at three sites in Colorado at various times during the growing season to see if the collection and disposal of reproductive stalks is necessary. I found that harvesting before seed capsules had turned brown reduced seed viability dramatically (mean early reproductive stage = 0.08%, 95% CI = 0.06%, 0.67%; mean mid reproductive stage = 1.52%, 95% CI = 0.49%, 3.11%). I suggest focusing mechanical control efforts during the early and mid season if possible, as at those times plants can safely be left on site. When control efforts occur later in the season after seed capsules begin to turn brown, it is important that reproductive stalks be removed from the field.

ACKNOWLEDGMENTS

I would first like to thank my committee, Ruth Hufbauer, Andrew Norton and Paul Meiman, for their aid and guidance during the last three years. They each assisted in expanding my understanding of invasion biology and management and the field of science in general all while assisting with my plans for the future and mentoring me in my path. Without them, this thesis and the work that it represents would not have been possible.

My advisor, Ruth Hufbauer was especially instrumental, and supported not only my research, but my teaching, my classwork, and my personal pursuits. Ruth always had her door open, and I could not have gotten through this process without her encouragement and help.

I would also like to thank all of the members of the Hufbauer lab: Chrissy Alba, Stacy Biddlecomb, Christa Fettig, Sabrina Kumschick, Steve Rauth, and Marianna Szucs. Their friendship, collaboration, and positive reinforcement helped me immensely. I am especially indebted to Chrissy and Christa, who were always ready to talk about research, classwork, or family.

I must also thank the GDPE community, as they are an amazing group of scientists, managers, and intellectuals. I also thank the GDPE community for all of the great friendships I have developed. I feel very lucky to have been a part of this program and to carry it with me into the future.

Finally I would like to thank my family, which has grown both in number and in love since I started at CSU. My parents, my new and future in-laws, and my siblings have all been an integral part of this process, and I am deeply grateful to them.

DEDICATION

To my husband Jon. Thank you for all of your dinners, foot rubs and hugs over the past three years. Your support has meant everything.

TABLE OF CONTENTS

ABSTRACT.....ii

ACKNOWLEDGMENTS.....iv

DEDICATION.....vi

THE EFFECT OF INSECT HERBIVORY ON THE PERFORMANCE OF
VERBASCUM THAPSUS L. IN COLORADO.....1

LIMITING COMMON MULLEIN (*VERBASCUM THAPSUS* L.) SEED OUTPUT VIA
EFFECTIVELY-TIMED HARVEST.....35

APPENDIX A.....49

THE EFFECT OF INSECT HERBIVORY ON THE PERFORMANCE OF
VERBASCUM THAPSUS L. IN COLORADO

Summary

A majority of the plant species that are introduced into new ranges either do not become established or become naturalized without becoming invasive. The biotic resistance hypothesis suggests that competition and herbivory reduce growth and reproduction of individual plants and so suppress population growth of non-native species. To test the effect of insect herbivory on the non-native biennial plant *Verbascum thapsus*, we followed a population in a disturbed habitat in Loveland, Colorado. Plants were either protected from herbivory with an insecticide treatment or were exposed to ambient levels of herbivory. Reducing herbivory had a marginally significant effect on survival rate, increasing survival to reproduction by 6%. Reducing herbivory increased plant diameter in the first year and plant height, the length of the reproductive spike, and seed set during the second year. Overall, protected plants produced an average of 37,975 (95% CI = 28,967, 49,370) seeds, while plants exposed to ambient herbivory produced an average of 18,944 (95% CI = 13,983, 25,201) seeds. However, the relationship between herbivory and performance is complex: among plants exposed to ambient herbivory, we observed a positive relationship between damage and performance suggesting that insect herbivores choose the largest plants for oviposition and feeding when their choice is not restricted by insecticide treatment. In contrast, there was no correlation between damage and

performance among plants that experienced reduced herbivory. Overall, we found that herbivore-induced reduction in individual plant performance and seed set may prevent this naturalized species from becoming dominant in undisturbed recipient communities.

Introduction

There are over 29 published hypotheses addressing the success of introduced species in their new range (Catford et al. 2009). Support for the different mechanisms is mixed, with experimental investigations offering conflicting results (Colautti et al. 2004, Catford et al. 2009). One reason for this discord could be that research has largely focused on species that have become dominant members of the community in their novel range or that instigate obvious and extensive environmental and economic costs (Hawkes 2007). However, the vast majority of introduced species are not dominant (Williamson and Fitter 1996), and many are considered benign (Lockwood et al. 2007, Dietz and Edwards 2006) or even beneficial (Schlaepfer et al. 2011) in their new range. This bias in research performed toward those species that cause obvious damage may obscure patterns that would be apparent if more data were available from the entire range of non-native species, spanning the spectrum from beneficial to detrimental.

To gain a better understanding of the mechanisms that enable some introduced species to become invasive, we must determine what mechanisms prevent other introduced species from becoming dominant. Investigating the effects of herbivory on non-native plant performance provides a unique perspective for understanding the spectrum of invasiveness among introduced species. In general, insect herbivory reduces growth, reproduction and survival of plants in their native ranges (Bigger and Marvier

1998, Carson and Root 1999, Maron et al. 2002, Maron and Crone 2006), and can regulate plant population sizes and community composition in the new range (Davidson 1993, Louda and Potvin 1995, Oliff and Richie 1998, Parker et al. 2006, Russell et al. 2010).

However, the ability of insect herbivores to regulate populations of non-native plants is variable (Keane & Crawley 2002) and is dependent on the plant species and recipient community (Suwa et al. 2010). Escape from herbivory often facilitates invasion (the case of enemy release; Elton 1958, Keane and Crawley 2002), but herbivore damage in the new range can also curb the growth of introduced plant populations (a component of biotic resistance; Maron and Vila 2001, Levine et al. 2004, Alpert 2006, Catford et al. 2009).

Recent work supports the dual roles of herbivory in shaping the outcome of invasions. In a meta-analysis, Hawkes (2007) compared herbivore damage in the native and introduced ranges of plants and found that the degree of invasiveness in the new range was directly correlated to damage from herbivory. Plants classified as noxious weeds (i.e. on the strongly invasive side of the spectrum) had lower levels of herbivory in the new range, while less weedy plants had similar levels of herbivory in the new and introduced ranges. Importantly, introduced populations of a plant may benefit from escaping herbivores even though they are suppressed to some degree by herbivores accumulated in the new range (Colautti et al. 2004).

In the research reported here, our broad goal is to contribute to the growing body of research on introduced populations of species that do not regularly dominate host communities and thus improve our understanding of the processes driving biological

invasions. We have focused on the effects of insect herbivory on performance of *Verbascum thapsus* (common mullein), an herbaceous biennial, in its introduced range due to its long residency and ample expansion in the new range while still not being considered a species of great concern.

Verbascum thapsus has been present for several centuries in the United States since its introduction from Europe. It is currently found in all US states and most Canadian provinces (USDA, NRCS 2011). Despite this significant range expansion, *V. thapsus* is restricted to disturbed habitats and is thus seldom considered a priority for limited management resources.

Recent evidence demonstrates that *V. thapsus* populations in the mountain west of the U.S. are more dense and larger, and individual plants are also larger than in the native European range. These changes in performance are associated with a decrease in insect herbivore species richness as well as a significant reduction in leaf-level herbivory of *V. thapsus* in the western United States relative to the native range, suggesting that introduced populations have escaped natural enemies and may experience release from top-down population regulation.

Substantial herbivory by generalists as well as co-introduced specialists does occur however in introduced populations (Alba and Hufbauer, in review). At some locations in Colorado, *V. thapsus* plants lose 25% of their leaf tissue to generalist grasshoppers and caterpillars (Alba, pers. comm). Additionally, at least two co-introduced herbivorous insect species are found in North America. *Gymnetron tetrum* (Fabricius), a specialist weevil, is found throughout the introduced range of *V. thapsus* (Buchanan 1937). The larvae develop in the maturing seed capsules and consume all

seeds within before emerging (Reinartz 1984). A second specialist (*Haplothrips verbasci* [Osborn]) is more common in the introduced range than in the native range (Alba and Hufbauer, in review). Thus, even though release from enemies may contribute to larger individuals and populations in the introduced range relative to the native range, herbivory does occur in the introduced range. We hypothesized that even the reduced level of herbivory found in the introduced range of the plant would significantly decrease plant performance.

The main objective of this research was to evaluate the effects of herbivory on performance of *V. thapsus* by experimentally reducing herbivory on plants in the field using insecticides. Specifically, we evaluated whether reduced herbivore damage increased survival to reproduction, performance during the first growing season (rosette size and biomass), performance in the second growing season (plant height, inflorescence length) and performance when protected from herbivory during both growing seasons (plant height, inflorescence length and seed production). To accomplish our main objective, we first needed to evaluate whether our insecticide treatments successfully reduced herbivory, and to test for experimental artifacts caused by either the addition of water to plants or by direct effects of the insecticide. Below, we discuss methodological issues first, then the results of experimental reduction of herbivory on *V. thapsus*.

If insect herbivores successfully regulate *V. thapsus* populations in Colorado, we would expect that plants will respond positively to a reduction in herbivory by growing taller, having longer inflorescences, and producing more seeds.

Methods

Study system

Verbascum thapsus is a monocarpic forb with an annual to triennial life cycle (Reinartz 1984), though it is a biennial through most of its range. It germinates in the early spring and forms a woolly-leaved rosette in the first year of growth. After overwintering in the rosette stage, plants send up a flowering spike that can reach up to 2 m in height (Baskin and Baskin 1981, Gross 1980). While the typical growth form is a single spike, particularly large plants and those that incur apical meristem damage may produce several axillary inflorescences (Lortie and Aarssen 2000).

Verbascum thapsus reproduces purely by seed. Seeds are small (50 to 100 μm) but plants are quite prolific; a single plant may produce more than 100,000 seeds in its lifetime (Gross and Werner 1978, Rutledge and McLendon 1996). Seeds can remain dormant in the soil for long periods before germination (Kivilaan and Bandurski 1981). *Verbascum thapsus* flowers from June to September in the western United States, with a few flowers along the stalk opening every day. The flowers of *V. thapsus* last for one day and will self-pollinate when closing if pollination by insects has not already taken place (Gross and Werner 1978).

Experimental design

We began our two-year study in April of 2009 by identifying 575 *V. thapsus* plants at a site in Loveland, CO (40°22'29" N, 105°13'32" W, elevation 1650 m) with a history of substantial disturbance, including being used as a staging area for a large construction project and for grazing. The site is currently owned by Larimer County and is maintained

as open space. We tracked both overwintered rosettes until they bolted ($n = 122$) and first year rosettes through their lifecycle ($n = 307$). Plants were mapped along 8 transects, marked with a raised nail and metal tag and assigned randomly to one of two treatments: reduced herbivory (insecticide) or ambient herbivory (water).

Plants were sprayed with either an insecticide solution or water every two weeks during the growing season using a 5-gallon Solo backpack sprayer. Applications were made in the evening to avoid chemical burning of the plants and to reduce the effect on pollinating insects. Additionally, we investigated whether the water added in the ambient herbivory treatment increased plant performance by comparing the water controls to 42 plants that received no treatment.

The first insecticide application was a mixture of esfenvalerate, (33112 Insecticide Concentrate, Bengal Products, Inc. 3.48% active ingredient esfenvalerate) which has been shown to have no or little effect on plant growth (Root 1996) and spinosad, (Lawn and Garden Spray with Spinosad, Green Light Company, 0.5% active ingredient spinosad). Spinosad is particularly effective against Thysanoptera, and thus was chosen to target the specialist thrips *H. verbasci*. We used a recommended rate of 0.0015% esfenvalerate and 0.0076% spinosad per 1 liter of water solution.

We used Bayer Advanced Dual Action Rose & Flower Insect Killer Concentrate with active ingredients imidocloprid (0.72%) and beta-cyfluthrin (0.72%) for all subsequent herbivory reduction treatments. Our own research did not uncover any effects of the insecticide mix on plant growth, (Appendix 1) and a longer study by Williams et

al. (2010) revealed no effects of imidocloprid on plant growth. The insecticide was applied at a rate of 0.0028% of imidocloprid and 0.0028% beta-cyfluthrin per 1 liter of water solution.

Herbivore damage

To evaluate the effectiveness of the herbivory reduction treatment and to gather data for inclusion in subsequent analyses, we estimated leaf herbivory experienced by each plant every month during both growing seasons. Herbivory was scored using Lewis et al.'s (2006) ranking system from 0 to 4, with 0 indicating no herbivory, and 4 being more than half of the plant affected (Table 1.1). We also evaluated our ability to reduce seed predators at the end of the 2010 season by comparing seed capsule attack rates between the two major treatment groups (see *Plant Performance 2010*).

Table 1.1. Herbivory scores used in the field to determine effectiveness of the treatments on reducing herbivory (Lewis et al 2006).

Damage score	Definition
0	No discernable herbivory
1	Minimal damage with no more than about 5% of any leaf damaged
2	Minimal damage plus 2 leaves with 5-10% damage
3	10-50% damage on 2 leaves, but fewer than half of all leaves affected
4	At least half of all leaves with 10-50% damage, and multiple leaves with more than 50% damage

Plant cover

Cover of plants surrounding target individuals may negatively affect growth via competition, or alternatively may be associated with higher quality sites and greater plant growth. To take these processes into account, we took digital photographs to estimate the percent cover of vegetation surrounding each plant during the first week of June in both 2009 and 2010. We used a digital camera at a height of 68 cm above the ground to estimate plant cover in a 30 by 30 cm area around first year rosettes and a 15 by 30 cm area in the cardinal directions around second year plants. These photos were used to measure percent cover of plant functional groups and percent bare ground with SamplePoint using methods described in Booth et al. 2006.

Plant Performance 2009

At the beginning of the growing season, before treatment, we measured initial rosette diameter in two directions and estimated levels of herbivore damage on the leaves. At the end of the growing season, we collected information on survival and growth of each of the plants.

For surviving first year rosettes, ($n = 343$) we measured rosette diameter, counted the number of leaves and took a final estimate of herbivore damage for the year. A subset of these surviving plants ($n = 58$) were harvested at the base of the plant, dried in drying ovens and weighed until their weight did not change from one time to another. We then weighed the dried rosettes to the nearest 0.1 g. We harvested all bolting plants as they

senesced in mid-September 2009. From each plant we collected data on plant height, inflorescence length (from the first seed capsule on the reproductive stalk to the top of the stalk), and number of branches.

Plant Performance 2010

In April of 2010 we identified all plants that had overwintered successfully and resumed treatment regimes. At the end of the growing season we measured plant height, inflorescence length and number of branches as outlined above. Inflorescences were collected from each of the plants for further study. Additionally, we measured seed capsule density, number of seeds per seed capsule, and estimated an overall number of seeds per plant.

We estimated the number of seed capsules per plant for each of the collected inflorescences by multiplying the density of capsules along a portion of the inflorescence by the total length. For density measures, the top 5 centimeters of the inflorescence was removed and all seed capsules on the next 20 cm of inflorescence were counted. We removed the top 5 centimeters of the inflorescence due to our observations that seed capsules at the top of the inflorescence were smaller and denser than the rest of the inflorescence and thus not representative of the inflorescence as a whole. When the inflorescence was shorter than 25 cm, the top 5 cm was still removed and the remaining seed capsules counted. We did not estimate the number of seed capsules on inflorescences shorter than 5 cm.

The specialist seed predator *G. tetrum* reduces the number of seeds to close to zero in an infested capsule (Reinartz 1984). As *G. tetrum* is found throughout the

introduced range of *V. thapsus*, we needed to evaluate the abundance of weevils within our treatments and the subsequent reduction in seedset that this would represent. We used all inflorescences longer than 20 cm ($n_{\text{ambient}} = 60$, $n_{\text{reduced}} = 46$) to estimate the proportion of seed capsules attacked by *G. tetrum*. The length of the inflorescence was divided into 5 intervals, minus the top and bottom 5 cm. At each of the intervals, all of the seed capsules within 2 cm were counted and then dissected to determine if weevils were developing inside.

In order to determine the average number of seeds produced per capsule, we collected individual seed capsules from a subset of the treated plants in the field ($n_{\text{ambient}} = 24$, $n_{\text{reduced}} = 24$). We removed seed capsules at equal intervals along the inflorescence for a total of 7 seed capsules per plant. Seed capsules were only collected if the capsule was closed and appeared to be undamaged. Each seed capsule was placed in its own envelope. To calculate the average number of seeds per capsule for an individual plant, we developed a seed mass to seed number ratio. With the ratio we then obtained an estimate of seed number per capsule by weighing 50 seeds from a capsule, then all of the seeds in the capsule. Weevil infestation lowered our subsampling within plants to one ($n = 23$ plants) or two ($n = 25$ plants) seed capsules per plant.

Ultimately, we were most interested in investigating how the treatments affected the number of seeds produced per plant as this is most closely allied with fitness. We estimated seed set per plant for those plants that were treated during both growing seasons. We calculated the number of seeds per plant as: (total inflorescence length) \times (seed capsule density) \times (proportion of capsules without weevils) \times (number of seeds per capsule). As we did not measure seed capsule density on all of the inflorescences, we first

estimated their density. An exploratory analysis revealed that density is influenced by inflorescence length such that longer inflorescences have more capsules per centimeter. Field observations suggest this is because longer inflorescences have a wider diameter, and thus have more space per unit length. To estimate density for plants on which it was not directly measured, we first identified the relationship between total inflorescence length and the density of seed capsules for those plants that had been included in our sampling ($n = 212$). Similarly, if the proportion of capsules without weevils or the number of seeds per capsule was not directly measured, we estimated this using the average for each treatment (as there were no significant relationships with inflorescence length). We performed these calculations for two sets of plants –for all plants that bolted, and for all plants included in the experiment. This second measure incorporates survival, with plants that died by definition having a seed output of zero.

Statistical Analysis

Herbivore damage

To assess the effectiveness of the insecticide treatment in reducing herbivory, we used a mixed model in JMP 9 (SAS, 2011). Separate ANOVAs were performed for each monthly sample period and for average damage scores for the 2 years. Treatment was included in the models as a fixed effect and transect as a random effect. We used the same model structure to evaluate whether treatment altered the proportion of seed capsules with weevils.

Effect of water treatment on plant performance

To evaluate the effects of water addition from insecticide treatments on plant growth, we used a mixed model to compare plants that had received the water-only treatment (ambient herbivory) to the untreated plants. We included treatment as a fixed effect and transect as a random effect. We performed separate ANOVAs for each of the response variables which included plant height, total inflorescence length, and seed capsules per plant.

Effect of reduced herbivory on plant performance

We used PROC GLIMMIX in SAS 9.2 with a binomial error distribution (alive vs. dead) to test for differences in survivorship among treatments. The model included treatment, initial plant size, vegetative cover, average herbivore damage and the interaction between herbivore damage and treatment as fixed effects and transect as a random effect. While the insecticide treatment was effective at reducing insect attack (see results below), levels of herbivore damage were highly variable within each treatment. Thus, it was also important to include a measure of herbivory as a fixed effect. Herbivore damage level was highly variable within treatments, but was on average reduced by the insecticide treatment. We tested explicitly for an interaction between treatment and herbivory. Significant differences between LS means using Tukey's adjustment were our determinants.

To evaluate the effects of herbivory on the performance of *V. thapsus* that survived, we created mixed models in JMP that included treatment (ambient vs. reduced herbivory), initial rosette area in 2009, vegetative cover, herbivore damage, and the

interaction between herbivore damage and treatment as fixed effects and transect as a random effect. For first year plants (measurements taken at the mid-point of the experiment) response variables analyzed using separate ANOVAs included final rosette size, number of leaves per rosette, and rosette biomass. For second year reproductive plants (measurements taken at the end of the experiment), the response variables analyzed using this model included plant height, inflorescence length, number of seed capsules per plant, number of seeds per capsule, and number of seeds per plant (with and without incorporating survival). The analysis of number of seeds per capsule also included location of the seed capsule on the inflorescence as a fixed effect.

For the above models, we log transformed total inflorescence length, estimated number of seed capsules, and initial and final area of rosettes and arcsine square root transformed percent germination and the percent of seed capsules with weevils to improve the normality of the residuals. To achieve normality of residuals for number of seeds per plant, we first added 5000 to the base estimate and then performed the log transformation (Sokal & Rohlf 1995). All data presented have been back-transformed for ease of interpretation.

Results

Herbivore damage

Damage did not differ between the plants assigned to the two treatments prior to the start of the experiment (Figure 1.1A), indicating no bias in treatment assignments at the outset. Subsequent insecticide application significantly reduced herbivory in 2009 (Figure 1.1B). In spring of 2010, the first damage estimates did not differ between treatment groups

(Figure 1.1C), but rates of herbivory were low at that time. By the second month, and on average throughout the experiment (Figure 1.1D), insecticide treatments were effective in reducing leaf herbivory.

Insecticide treatment also reduced attack by the seed-feeding weevil *G. tetrum* ($P = 0.0483$). The reduction was modest: plants with ambient levels of herbivory had weevils in 26.3% of seed capsules (95% CI = 21.9%, 31.1%), while those with reduced herbivory had weevils in 21.7% of seed capsules (95% CI = 17.5%, 26.1%).

Effect of water treatment on plant performance

Plants in the ambient herbivory (water-only) treatment did not differ from untreated controls with respect to plant height ($F_{1,155} = 0.3454$, $P = 0.5576$), total inflorescence length ($F_{1,157} = 0.4545$, $P = 0.5012$), or seed capsules per plant ($F_{1,147} = 0.0053$, $P = 0.942$).

Effect of reduced herbivory on plant performance

Plant survival through time

Reducing herbivory in either the first or second growing seasons alone did not affect plant survival ($P = 0.25$, $P = 0.7812$, respectively). However, over the full life cycle of the plant there was a modest difference in survival between the two treatments (mean_{water} = 83.9%, 95% CI = 75.0%, 92.8%, mean_{insecticide} = 90.5%, 95% CI = 84.7%, 96.3%, $P = 0.0788$).

Area, leaf number, and biomass of first-year rosettes

Plants in the reduced herbivory treatment had larger rosettes at the end of the first growing season by 20.13% (Figure 1.2A), but did not have more leaves (Figure 1.2B) or greater biomass (Figure 1.2C) than plants in the ambient herbivory treatment. While there were not significant interactions between herbivore damage and treatment, plants with higher average herbivore damage had larger final area ($F_{1,334} = 4.2955$, $P = 0.039$). Both final area and number of leaves were positively related to initial area ($F_{1,336} = 11.5172$, $P = 0.0008$, $F_{1,337} = 26.503$, $P < 0.0001$). Vegetative cover did not affect final area, number of leaves per plant or biomass ($F_{1,336} = 2.4966$, $P = 0.1150$, $F_{1,330} = 0.0156$, $P = 0.9007$, $F_{1,63} = 0.0490$, $P = 0.8256$).

Growth and reproductive output of bolted plants

Experimentally reducing herbivory increased plant performance relative to the ambient herbivory controls. This was true for plants treated only during their second growing season (2009 bolting plants) and during both growing seasons (2010). Specifically, reducing herbivory in only the second growing season (plants that bolted in 2009) increased plant height by 9.41% (Figure 1.3A) but did not influence total inflorescence length (Figure 1.3B). Over two years, the herbivory reduction treatment increased plant height by 17.25% ($F_{1,237} = 8.5016$, $P = 0.0039$, Figure 1.4A), total inflorescence length by 57.75% ($F_{1,235} = 20.3818$, $P < 0.0001$, Figure 1.4B), and number of seed capsules per plant by 72.28% ($F_{1,204} = 12.6826$, $P = 0.0005$, Figure 1.4C). The number of seeds per seed capsule remained comparable between treatments ($F_{1,63} = 1.4336$, $P = 0.2357$, Figure 1.4D). Focusing just on plants that survived and produced an inflorescence, the average number of seeds per plant increased 70.08% when protected from herbivory

($F_{1,237} = 7.9591$, $P = 0.0052$, Figure 1.5A). Expanding that analysis to include plants that did not survive (and thus produced no seed) the average number of seeds per plant increased 100.45% when protected from herbivory ($F_{1,313} = 20.1469$, $P < 0.0001$, Figure 1.5B).

For many of these traits, there was a significant interaction between mean herbivore damage levels and treatment. There was no significant relationship between performance measures and damage levels in the reduced herbivory treatment, but in the ambient herbivory treatment greater damage was associated with greater performance. The interaction between treatment and herbivore damage was significant for both plant height ($F_{1,113} = 15.3719$, $P = 0.0002$, Figure 1.6A) and inflorescence length ($F_{1,115} = 5.8529$, $P = 0.0171$, Figure 1.6B) among bolting plants in 2009. The interaction between treatment and herbivore damage was significant for plant height ($F_{1,235} = 7.0388$, $P = 0.0085$, Figure 1.7A) seed capsules per plant ($F_{1,201} = 11.8143$, $P = 0.0007$, Figure 1.7B), seeds per bolting plant ($F_{1,235} = 12.468$, $P = 0.0005$, Figure 1.8A), and seeds per plant incorporating survival estimates ($F_{1,313} = 15.8325$, $P < 0.0001$, Figure 1.8B) in 2010.

In 2009, plants with larger initial area in the spring grew taller by the end of the growing season ($F_{1,68} = 142.1229$, $P < 0.0001$) and had longer inflorescences ($F_{1,111} = 102.0338$, $P < 0.0001$). At the end of two years, plants with larger initial area at the beginning of the experiment were also taller and had longer inflorescences ($F_{1,239} = 4.1349$, $P = 0.0431$, $F_{1,236} = 4.7977$, $P = 0.0295$).

Inflorescence length was positively affected by increasing vegetative cover among plants that bolted in 2009 ($F_{1,115} = 4.8277$, $P = 0.0300$).

Discussion

We explored the role that insects might serve in regulating the performance of introduced *V. thapsus*. Although *V. thapsus* has escaped from several herbivores following introduction and there is indirect evidence of enemy release, (Alba and Hufbauer, in review) it does incur substantial attack in the introduced range. This agrees with the findings of Colautti et al. 2004 that reductions in enemy pressure are common at the biogeographic scale, but that they do not necessarily translate into a fitness advantage over native plants growing in the introduced range.

Given that *V. thapsus* mainly populates disturbed rather than intact landscapes in its new range, it provides an opportunity to explore whether herbivores have prevented it from becoming a problematic weed. Indeed, we found evidence that herbivory by insects affects several aspects of growth and reproduction of *V. thapsus* in the introduced range including plant survival, overwintering rosette size, plant height, inflorescence length, seed capsules per plant, and the number of seeds produced per plant.

Effect of reduced herbivory on plant performance

Plant survival through time

Because *V. thapsus* is semelparous, the most effective form of biotic resistance would prohibit survival to reproduction. We found that plants experiencing less herbivory had a 6% higher chance of survival overall. While only marginally significant, that a difference in survival can be detected is of note given the damage on the insecticide-treated plants. If we had been able to successfully eliminate rather than reduce herbivores, we potentially could have seen an even greater difference in survival.

Area, leaf number, and biomass of first-year rosettes

Increased performance associated with reduced herbivory was apparent even in a single growing season. First-year rosettes experiencing reduced herbivory grew larger than those exposed to herbivory. Larger overwintering rosettes produce taller plants in the next season, and so by reducing herbivory during the rosette stage of the plant, we increased the future height of the plants. This in turn affects the number of seeds produced per plant (see below).

Growth and reproductive output of bolted plants

Reducing herbivory on bolting plants in 2009 did not affect the length of the reproductive spike, but it did significantly increase overall plant height (Figure 1.3). This significant difference in plant height can cause a change in overall seed set of the plants by altering ecological interactions with pollinators in the introduced range. The flowers of taller plants are more apt to be pollinated (Carronero and Hamrick 2005), and seed capsules from cross-pollinated flowers produce more seed than seed capsules from self-pollinated flowers (Donnelly et al. 1998). As such, the reduced height that results from herbivory could further decrease fitness of plants.

Reducing herbivory for two growing seasons revealed that insect herbivory inhibits individual growth of *V. thapsus* by reducing plant height, inflorescence length, and seed capsule number which together causes a reduction in the number of seeds produced per plant. Modeling carried out by Maron and Gardner (2000) predicts that reducing seedset of long-lived seeds has the potential to reduce population size in the future. Given that mullein produces large and long-lived seed banks, an additive reduction in seeds entering the soil could diminish the number of propagules available for

recruitment. However, others have found that a reduction in seedset caused by insect herbivores does not limit the number of propagules enough – seedset must be reduced beyond the population restrictions caused by density-dependent mortality after germination (Garren and Strauss 2009). We did not address density-dependent seedling mortality during our field experiment, but our observations suggest it could be quite important to population size regulation.

Interaction between patterns of herbivory and experimental treatments

We have shown that reducing herbivory on introduced *V. thapsus* directly affects several plant performance metrics, but have also uncovered a quite interesting pattern among plants experiencing ambient levels of herbivory. We detected a significant interaction between our experimental treatments (reduced versus ambient herbivory) and herbivory in association to performance. Herbivory was lower among plants in the reduced herbivory treatment on average, and was more equally distributed across both taller and shorter plants. In the ambient herbivory treatment, however, there was a positive relationship between damage level and several measures of plant performance (Figs 1.6, 1.7, 1.8).

Overall this positive correlation between herbivory and plant size suggests that, when unmanipulated, herbivores track plant performance rather than driving it as suggested by the plant vigor hypothesis (Price 1991). Among unprotected plants our data suggest that herbivores choose to oviposit and feed on the larger, more apparent plants (Cornelissen et al 2008). When plants were treated with insecticide, choice was reduced, and, in our experiment, it may have been less-mobile herbivores causing the damage.

Therefore, while the natural patterns of herbivore choice would cause larger, more apparent plants to be more damaged, we altered the choice regime.

Biotic resistance of Verbascum thapsus in the introduced range

Expanding current work in invasion biology to include less abundant and less widespread species is necessary so that we may have a better understanding of the effectiveness of native competition and predation on non-native plants. Our work adds to the sparse experimental information available for non-native plants in the middle of the spectrum between benign and strongly detrimental. It also lends insight into the importance of herbivory to this group of non-native plants. It appears that herbivory is having negative effects on *V. thapsus* by reducing survival, plant size and seed number in bolting plants. This, along with evidence of the weak competitive ability by *V. thapsus* (Gross 1980, Gross and Werner 1982) may contribute to the fact that it is only dominant in disturbance prone habitats despite reproducing prolifically. While populations of *V. thapsus* have spread throughout North America, our data suggest that there are population controls in place that have kept the species from becoming more ecologically harmful.

Figures

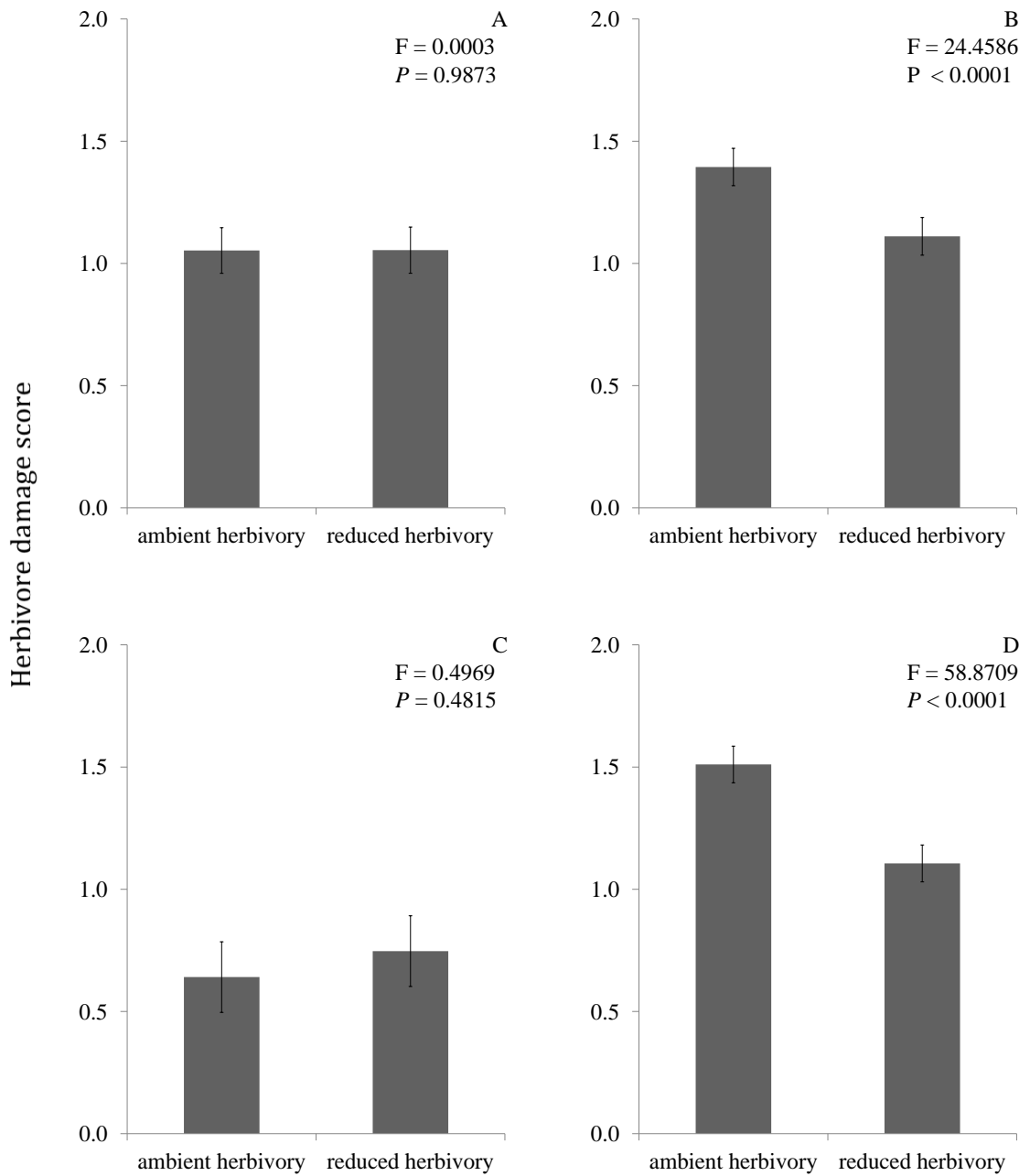


Figure 1.1. Average estimates of herbivore damage for A) May 2009 and C) April 2010 and yearly averages for B) 2009 and D) 2010. See Table 1.1 for details of herbivory scores.

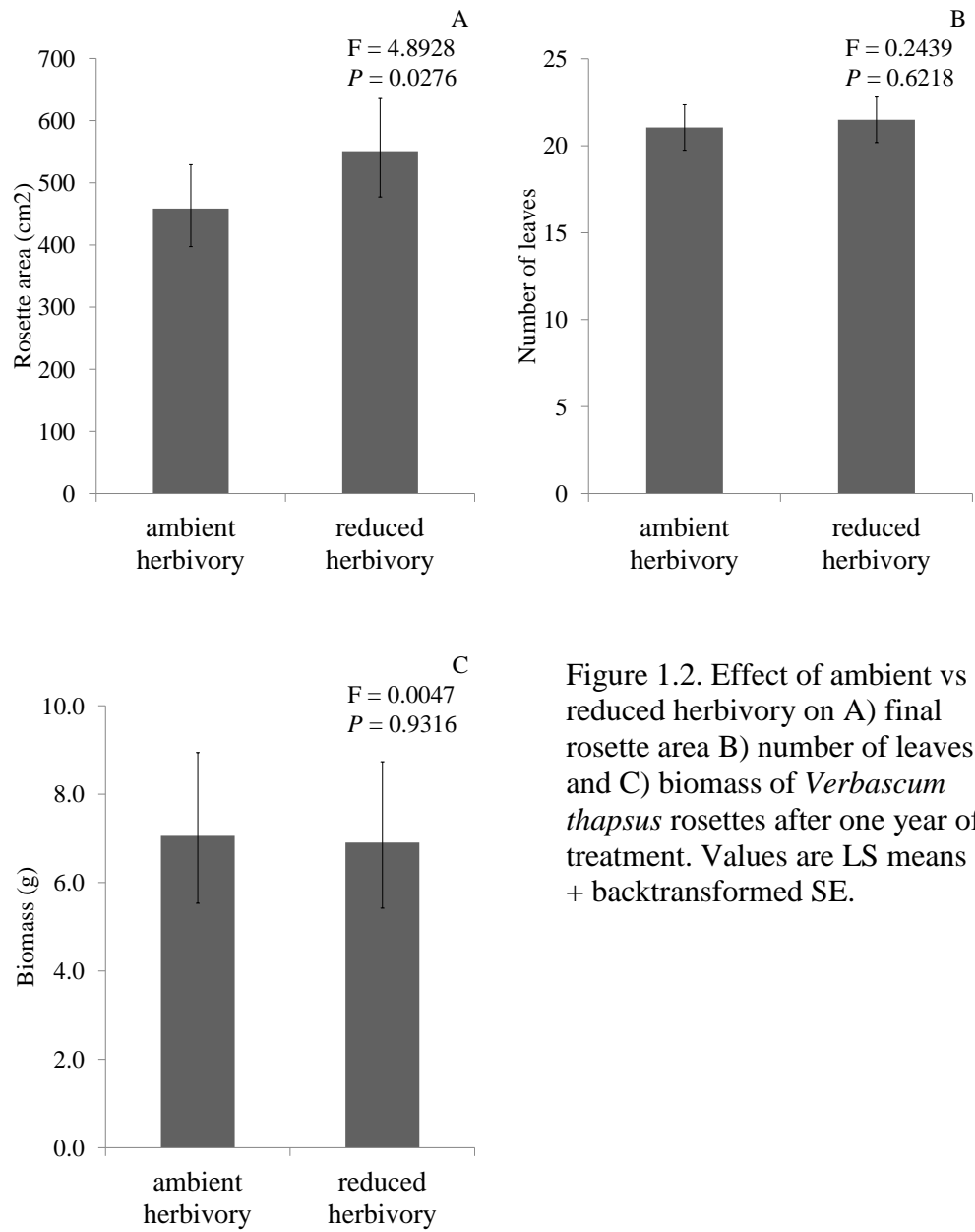


Figure 1.2. Effect of ambient vs reduced herbivory on A) final rosette area B) number of leaves and C) biomass of *Verbascum thapsus* rosettes after one year of treatment. Values are LS means + backtransformed SE.

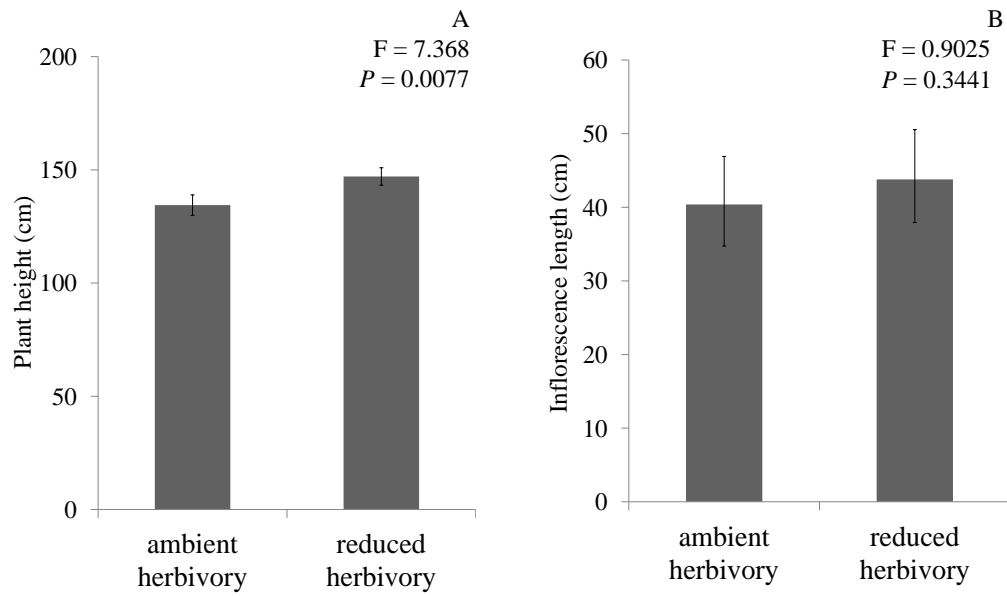


Figure 1.3. Effect of ambient vs reduced herbivory on A) plant height and B) total inflorescence length of bolting *Verbascum thapsus* after one year of treatment. Values are LS means + backtransformed SE.

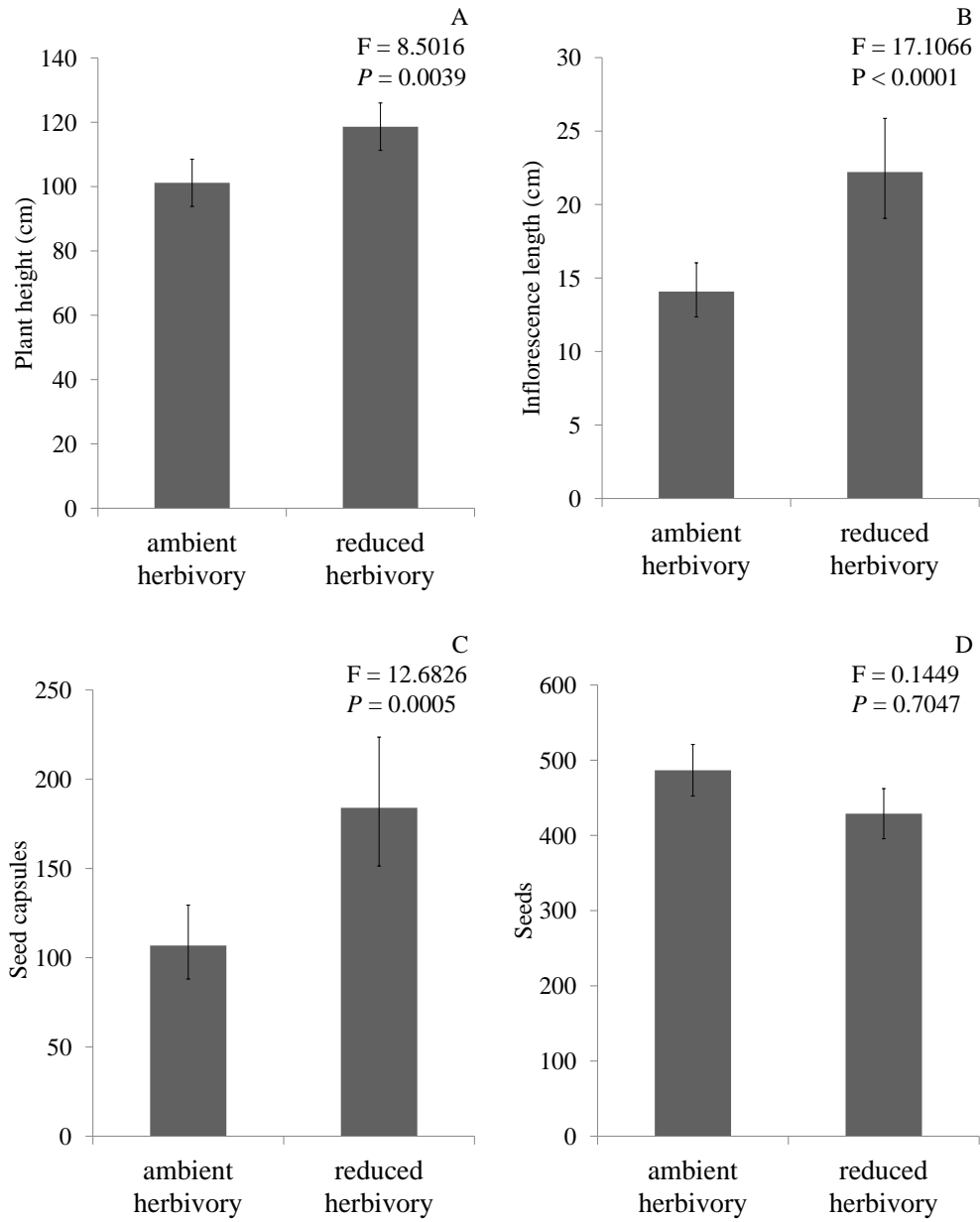


Figure 1.4. Effect of ambient vs reduced herbivory on A) plant height B) total inflorescence length C) estimated number of seed capsules and D) seeds per capsule of bolting *Verbascum thapsus* after two years of treatment. Values are LS means + backtransformed SE.

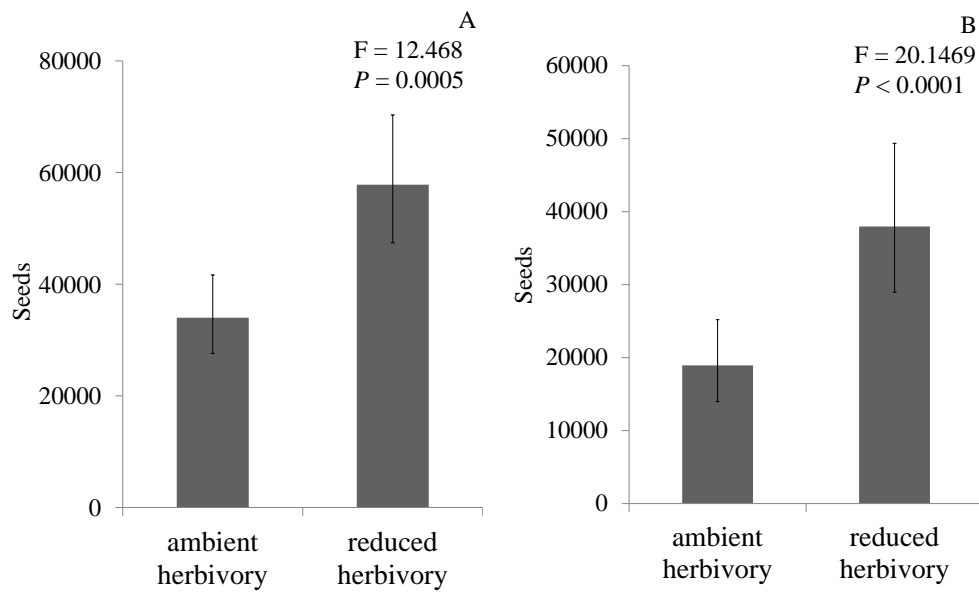


Figure 1.5. Effect of ambient vs reduced herbivory on A) seeds per bolting plant B) seeds per plant incorporating survival for *Verbascum thapsus* after two years of treatment. Values are LS means + backtransformed SE.

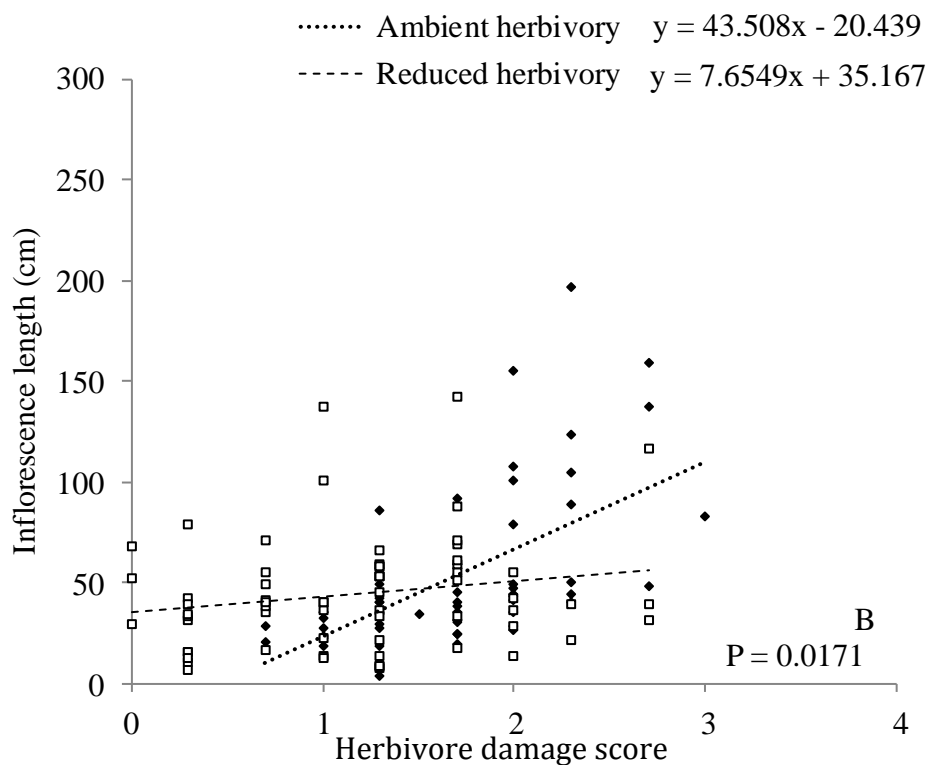
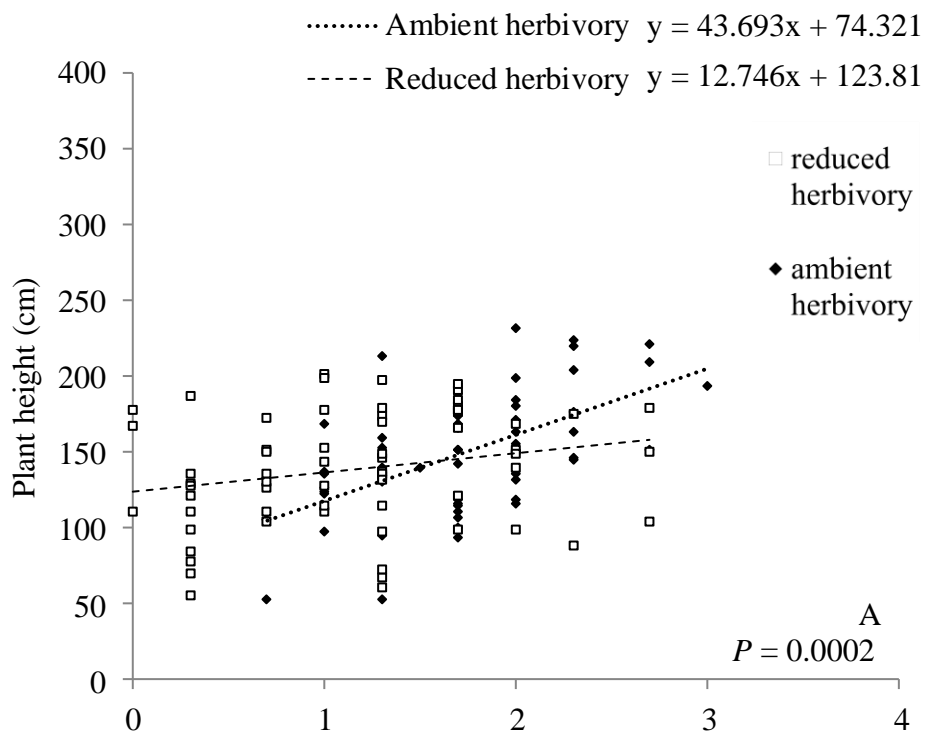


Figure 1.6. Interaction between herbivory damage and treatment on A) plant height B) inflorescence length of bolting *Verbascum thapsus* after one year of treatment.

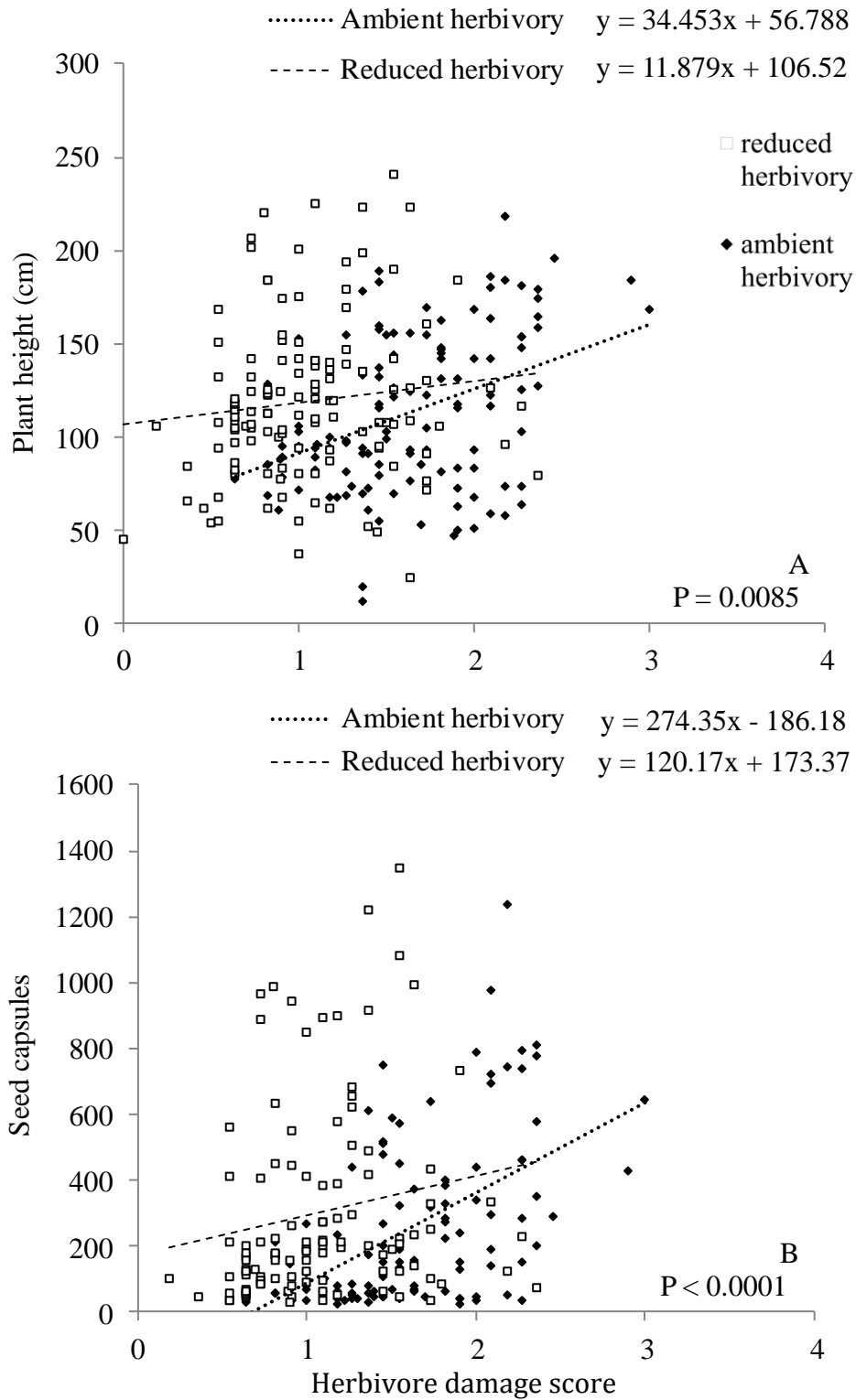


Figure 1.7. Interaction between herbivory damage and treatment on A) plant height B) estimated number of seed capsules of *Verbascum thapsus* after two years of treatment.

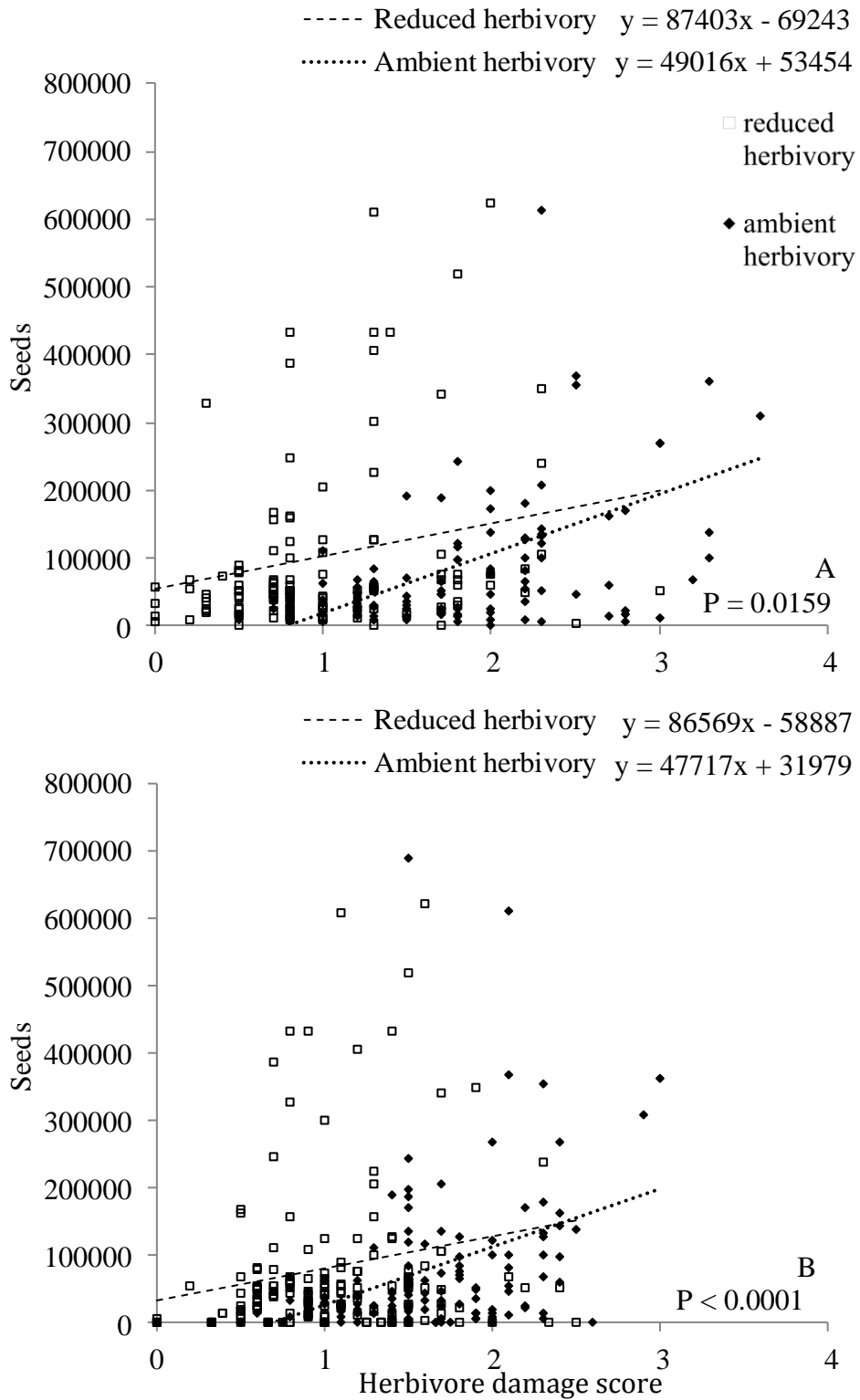


Figure 1.8. Interaction between herbivory damage and treatment on A) seeds per bolting plant B) seeds per plant incorporating survival of *Verbascum thapsus* after two years of treatment.

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LIMITING COMMON MULLEIN (*VERBASCUM THAPSUS* L.) SEED OUTPUT VIA EFFECTIVELY-TIMED HARVEST

Summary

Control options for the non-native plant common mullein are of increasing interest to land managers in the west. Common mullein is a prolific seed producer with a single plant able to produce well over 100,000 seeds. We found that mechanical control of common mullein before mature seed capsules have developed along the raceme significantly reduces viable seed set. Seeds from immature seed capsules have very low rates of viability (early reproductive stage = 0.08%, 95% CI = 0.06%, 0.67%; mid reproductive stage = 1.52%, 95% CI = 0.49%, 3.11%). Having this information allows managers to reduce the amount of material that must be disposed of in order to control the spread of seeds.

Introduction

Managing non-native plants in natural areas requires balancing tradeoffs involving time, expense, and efficacy while minimizing damage to desirable species. There are four main categories of management options: chemical, biological, cultural, and mechanical (Anderson et al. 2003, DiTomaso 2000). Chemical controls such as herbicides can be quite effective but are expensive and have ramifications for human and environmental

health (Crone et al. 2009, Mack et al 2000, Masters and Sheley 2001, Ortega and Pearson 2011). Classical biological control, the use of introduced enemies to control populations of exotic pests, can provide outstanding weed control to the benefit of native plants (Culliney 2005, Larson et al. 2008). However, development of a biological control program is costly and takes many years, and there are risks of indirect effects (Cory and Myers 2000) and damage to non-target species (Louda and Stiling 2004). Cultural controls such as prescribed burning, grazing, and revegetation of infested areas (DiTomaso 2000, Hendrickson and Lund 2010) can be successful when applied appropriately, but these approaches are also expensive and difficult to implement (Grant et al 2010, Perry et al. 2009). Hand pulling, tilling, mowing, or other physical removal efforts are forms of mechanical control (Masters and Sheley 2001). Mechanical control is laborious, but in most cases does not require complex or expensive equipment, and can be an excellent tool for engaging stakeholders (DiTomaso 2000, pers. obs.). It also does not have the inherent risks associated with a biological or chemical control.

We studied an approach for the mechanical control of common mullein (*Verbascum thapsus* L.), a common weed of disturbed areas. Common mullein reproduces exclusively by seed, and thus current practice is for land managers to cut off the inflorescences to reduce seed set. However, it is possible that developing seeds will successfully mature even if separated from the rosette. In direct response to land manager requests for input on this issue, we evaluated how the timing of seed capsule removal from a plant influenced subsequent seed viability.

Common mullein

Common mullein is a weedy herbaceous plant that is native to Eurasia and has been naturalized in North America for several centuries (Gross and Werner 1978). It is semelparous and follows a biennial lifecycle throughout most of its range (with annual forms found further south, and triennials occurring in the northern range limits, Reinartz 1984). In mid-temperate latitudes where it is biennial, seedlings germinate in the spring and develop into rosettes. In the second growing season, an inflorescence forms that can reach up to 2 m in height (Gross 1980, Reinartz 1984, Whitson 1996).

Each flower along the inflorescence develops into a two-halved capsule that can house as many as 500 small seeds. Individual plants may at any one time have mature seed capsules with a brown and brittle casing, immature seed capsules with a green and pliable casing, and open flowers. More mature seed capsules are generally found lower on the plant (Gross and Warner 1978, Reinartz 1984, Whitson 1996). Seed limitation is likely rare for common mullein, as a single healthy plant may produce 136,000 (Gross and Werner 1978) to 223,000 (Stevens 1932) seeds in its lifetime. Furthermore, seeds can remain viable in the seed bank for at least 100 years (Kivilaan and Bandurski 1981).

Individuals are most abundant in disturbed areas (Guennel 2004). Previous work on common mullein in Eastern Canada, South Carolina, and Texas predicts that a population will develop quickly after a disturbance, but within 3 to 4 years the number of individuals will be drastically reduced as other species colonize space around the plants (Reinartz 1984). In contrast, in drier climates in the western United States where open ground is plentiful, disturbance occurs more often, and vegetative regrowth can be slower, common mullein can be the dominant species in a system for much longer

periods of time. Stands can cover hundreds of acres and persist for almost a decade after disturbance (personal observation, Fornwalt et al. 2010). For example, 5 years after a large fire in central Colorado, the abundance of common mullein is increasing in the fire-disturbed areas (Fornwalt et al. 2010).

Control efforts

In Colorado, where our study areas are located, common mullein is a Class C noxious weed which indicates that there is a legislated need for an effective integrated weed management plan. With both high seed production and long-term viability in the seed bank, a dramatic reduction in seed set of mullein is needed to reduce subsequent populations.

Managers are faced with choosing a method to directly control common mullein. A thick layer of trichomes on leaf surfaces protects plants from treatment with herbicides (Carpenter and Murray 2000), making chemical control more difficult than for many other species. Biological control has not been developed for this system, and the risks may not be warranted for a species that dominates ecosystems relatively infrequently. Cultural control options such as fire and grazing will increase disturbance, and so facilitate germination of common mullein (Beck et al. 2004, Carpenter and Murray 2000, Pitcairn 2000). Given these constraints, mechanical control is the method of choice.

Digging or pulling up plants is considered the most effective management approach for common mullein (C. Landis, pers. comm.; Pitcairn 2000). The labor involved includes not only removing the plant from the ground, but also collecting flowering plants for disposal in a landfill. It is not known if seeds within immature

capsules are able to develop after plants are harvested. If they do not successfully complete their development, then there would be no need to bag and carry away the plant stalks. To evaluate whether managers may safely leave flowering stalks that have been pulled, we investigated whether seeds removed from plants before and after the capsules have matured continue to develop and produce viable seeds.

Table 2.1. Site names, nearest town, and geographic location of sites used.

Site name	Location	Latitude	Longitude	Altitude
Bobcat Ridge	Masonville, CO	40°28'33.31"N	105°14'7.49"W	1700 m
Horsetooth Mountain Park	Fort Collins, CO	40°31'29.56"N	105°10'41.86"W	1800 m
Hewlett Gulch	Poudre Park, CO	40°41'18.93"N	105°18'28.95"W	1700 m

Materials and Methods

During the summer of 2009, we collected seed capsules from bolting plants at three sites in northern Colorado, USA (Table 2.1). We focused on individual seed capsules rather than whole inflorescences so that we could classify them into two categories: mature (brown and brittle, but not yet opened) and immature (green). Seed capsules open and readily release their seeds as they dry, and so only by collecting individual seed capsules we could verify the origin of the seeds, given that inflorescences contain capsules at both stages.

At each of the three sites, seed capsules were collected from 10 plants per site four times during the flowering season. We collected during the early reproductive stage (July), while much of the inflorescence was still in flower and the few seed capsules present were immature; at a mid reproductive stage (August), when many seed capsules were present but none were mature; during the late reproductive stage (September), when

both mature and immature seed capsules were present; and post-reproduction (October), after plant senescence but before first snowfall, when all seed capsules had matured and many had split open (Table 2.2). Plants were selected haphazardly at each site, and after the seed capsules were harvested, the plant was clipped so that it would not be used again in subsequent collections. Capsules were taken from the lower end of the inflorescence so that the most mature seeds were collected.

Table 2.2. Timing of seedpod removal by reproductive stage classification, month, and phenology.

Reproductive Stage	Month of Removal	Phenology
Early	July	Immature seedpods collected, many flowers present
Mid	August	Immature seedpods collected, no mature seedpods present
Late	September	Mature seedpods collected, both mature and immature seedpods on plant
Post	October	Mature seedpods collected, after plant senescence

At the end of the growing season, seed capsules were kept at room temperature for one month to dry, then cold treated at -15 degrees C for one week to vernalize the seeds (Baskin and Baskin 1998). Then were they stored at 4 degrees C until used for germination on August 30, 2010.

Five seed capsules per site were used from each of the four reproductive stages. Seed capsules were opened and seeds examined via dissecting microscope to evaluate their development. Undeveloped seeds were defined as those that were completely flat, and did not have the bullet-shaped appearance of seeds in which an endosperm had

developed. Preliminary testing of these seeds revealed that they would not germinate, and so we were able to classify these seeds as non-viable.

For those seed capsules with developed seeds, we counted 50 seeds from each seed capsule then placed them in petri dishes on blotter paper wetted with 1 mL of distilled water. These were sealed with Parafilm and placed in a germination chamber for three weeks under an alternating temperature and light regime (light for 12 hrs at 25°C, and dark for 12 hrs at 15°C). An additional 1 mL distilled water was added after 1 week, and the dishes were resealed with Parafilm.

At the end of three weeks, we counted the seeds that had germinated, defined by the protrusion of a radical. The viability of seeds that had not germinated was evaluated using tetrazolium (Peters 2005). The seeds were soaked in water for 24 hours, cut down the middle and submerged in the tetrazolium solution for 24 hours at 30 degrees C. Seeds displaying a pink color after exposure to the tetrazolium solution were considered viable, while those where the material inside the seed was white were considered nonviable.

The total proportion of viable seeds was calculated in one of two ways. First, any seeds that were undeveloped were considered non-viable. Then, for those seeds that had developed, we added the number of seeds that germinated readily to the number of seeds that tested positive to tetrazolium for viability, and divided that by the total number of seeds in the petri dish.

We used analysis of variance (JMP 9.0.0) to evaluate how reproductive stage influenced the proportion of viable seeds. Location was included in the model as a covariate. Means were compared using Tukey's HSD. We also analyzed the effect of plant height on proportion of viable seeds within each collection period using analysis of

variance, as taller common mullein are known to attract more pollinators (Lortie and Aarssen 1999). Proportion viable was arcsine square root transformed before analysis to improve the normality of the residuals. After this transformation, residuals did not conform perfectly to the assumption of normality inherent to ANOVA, but the deviation was minor and ANOVA is known to be robust to violation of that assumption (Lindman 1974). The results are presented after back-transformation.

Results and Discussion

The timing of seed capsule removal by reproductive stage strongly influenced seed viability ($F_{3,45} = 198.211$, $P < 0.0001$). In contrast, viability did not differ by site ($F_{2, 45} = 0.3790$, $P = 0.6864$).

The mean proportions of viable seeds can be broken into two biologically distinct groups: early reproductive stage with mid-reproductive stage, and late reproductive stage with post-reproduction. Seed capsules collected in the early and mid reproductive stages had a very low proportion of viable seeds. In contrast, most of the seeds collected during the late and post reproductive stages were viable (Figure 2.1).

Plant height was associated with increase in seed viability in the late season harvest at a significance level of $P = 0.0521$ ($R^2 = 0.26$), but was not significant for the other three harvests.

Controlling common mullein populations is most effective when the plant is still in the rosette form, but removal of entire plants creates an opening for that may again be filled by common mullein seedlings (Pitcairn 2000). Moreover, the plant becomes very apparent after bolting, and may not be identified until then. Cutting the reproductive

stock and damaging the plant at the root will deter resprouting after initial control (Hoshovsky 2003). Our findings indicate that if the plant does not yet have brown seed capsules, the stocks can be left in the field and the seeds are unlikely to become viable.

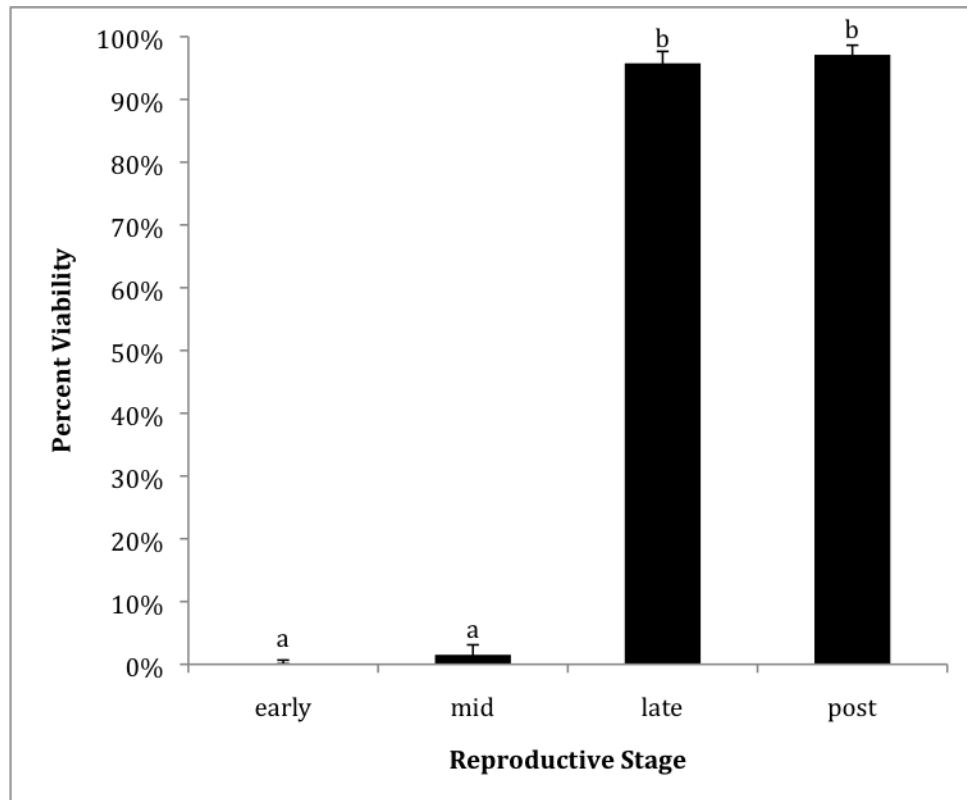
In contrast, plants harvested after seed capsules are brown should be collected and disposed of to reduce the number of viable seeds in the seed bank. While this approach is time consuming, it will contribute to reducing both the current population and the future seed bank.

We removed individual capsules from the plant to be able to confidently assign seeds to capsules of differing maturity. This may have influenced our results towards lower rates of viability, if green capsules attached to the inflorescence are able to successfully develop seed even after the inflorescence is removed from the plant. This could bias our results towards lower rates of viability. Should this be of concern, the conservative timing for management would be early in the flowering season (July in Colorado).

Common mullein is naturalized in the American west, and spreads without active assistance from humans. The reproductive strategy of common mullein allows it to rapidly expand its population size after disturbance. Controlling populations of the plant will keep the seed bank from growing and potentially facilitate the growth of native species in its place.

Figures

Figure 2.1. Percent viability of seeds by reproductive stage (Table 2). Significant differences at $P < 0.0001$ are indicated by different letters.



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APPENDIX A INSECTICIDE TRIALS

Introduction

We conducted a greenhouse experiment during the summer of 2009 to assess the effect of the insecticide Bayer Advanced Dual Action Rose & Flower Insect Killer Concentrate on the growth of *Verbasum thapsus*. Plants grown in a controlled environment were exposed to either an insecticide or a water spray over the course of a month.

Methods

During the fall of 2008, we collected inflorescences from 10 *V.thapsus* plants from a field site in Loveland, CO. We dried the inflorescences for one month at room temperature then harvested 10 seed capsules from each of the stalks. The seeds were cold treated at -15 degrees C for two weeks, then were kept in the refrigerator at 4 degrees C until ready for use (Baskin and Baskin 1998).

On July 1, 2009, the seeds were sown in plugs of Sunshine Mix #2 by Sun Gro Horticulture Canada Ltd. treated with fungicide. After one month we transferred the plugs to 4.5 x 4.5 cm x 6 cm pots of Turface Athletics MVP Sports Field Conditioner by Profile Products. Eight siblings from each of 10 parent plants were transplanted for a total of 80 plants. The plants were fertilized and kept in the greenhouse for one week after transplant, after which they were moved to a shaded outdoor area. They were watered and

fertilized as needed. Before treatment, all plants were treated with a 1% concentrate soap spray to remove any insects and hence reduce the possible positive effect of the insecticide treatment via herbivory reduction. Plants were randomly assigned one of two treatments: insecticide ($n = 38$) or water ($n = 30$).

As in the field, we used a rate of 0.39% of Bayer Advanced Dual Action Rose & Flower Insect Killer Concentrate (active ingredients 0.72% Cyfluthrin, 0.72% Imidocloprid) in a water solution for our insecticide treatment. For both the insecticide solution and for the water treatment, plants were treated with the same water as was used in the field.

Plants were sprayed until wet but not dripping in a spray chamber and then moved to a greenhouse bench. The plants received a second treatment two weeks later. All plants were harvested two weeks after the second treatment and dried for one week before being weighed.

We used a generalized linear model in JMP (9.0.0) to test for a difference in biomass between the insecticide treated plants and the water treated plants grown in the greenhouse.

Results

We did not observe any physical effects of the insecticide treatment on plant health. Plants were treated in the evening, and did not appear to experience any chemical burning. We did not find a difference in biomass between treatments for those plants grown in the greenhouse. The insecticide treatment did not appear to have either a negative or positive effect on plant growth ($P = 0.6575$, Figure A.1).

Figures

Figure A.1. Plant biomass by treatment.

