DISSERTATION

ECOLOGY AND PLANT DEFENSE OF TWO INVASIVE PLANTS, *HYOSCYAMUS NIGER*

AND *VERBASCUM THAPSUS*

Submitted by

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ABSTRACT

ECOLOGY AND PLANT DEFENSE OF TWO INVASIVE PLANTS, *HYOSCYAMUS NIGER*

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Understanding the factors that drive non-native plant populations to succeed in a new range and the ecological and biological differences that set introduced populations apart from their native counterparts can provide insight into ecological and evolutionary processes, as well as information crucial to management. In this dissertation, I present research on two different plant species that have been introduced to North America, both of which can now be found across the United States and throughout Canada.

Chapters 1 and 2 focus on *Hyoscyamus niger* (black henbane, Solanaceae), a poisonous and state-listed noxious weed. In chapter one I experimentally evaluate whether introduced populations in the western United States are annual or biennial. Both of these life cycles are found in the native range, and have a clear genetic basis. I experimentally manipulated vernalization (a cold treatment for 19 weeks), and find that plants in the introduced range are biennial. Vernalization is critical for bolting and flowering to occur within a growing season. Interestingly, given enough time in a greenhouse setting, 26 percent of plants that were not vernalized were able to flower. This is unlikely to happen in nature, however, as warmer regions without a cold period to naturally vernalize plants are typically lacking sufficient resources (e.g. adequate water or space) for this species. Chapter two aims to understand basic biological and ecological characteristics of black henbane in the introduced range, which lays the groundwork for additional ecological and evolutionary research on this species and will also help direct

appropriate management practices. In a greenhouse experiment, I test the effects of selfing and outcrossing. In field populations, I measure reproductive output, the size of seed banks of introduced populations, the viability of seed collected over four years, patterns of mortality, and fluctuation in the size of 15 populations. Black henbane is self-compatible, and capable of producing copious seed, and generating large seed banks in naturalized populations. Seeds remain viable for multiple years which may contribute to the dynamic fluctuations of field population sizes that were observed over four years. Populations are generally ephemeral, with high mortality at the rosette stage.

Chapter 3 is focused on resistance and tolerance to herbivory, and how they might vary between ranges as well as within individual plants as predicted by optimal defense theory. Optimal defense predicts that defenses are allocated to different tissues based on their value to the plant. I use *Verbascum thapsus* (common mullein, Scrophulariaceae) to evaluate resistance to both a specialist and a generalist herbivore among plants from the native and introduced range and among leaves of different ages. I also measure tolerance to defoliation by simulating three levels of herbivory and evaluating the regrowth of above and below ground biomass. Both native and introduced mullein plants are highly defended against specialist and generalist herbivores, with high levels of both resistance and tolerance. In accordance with optimal defense theory, young leaves are more highly defended than older leaves.

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CHAPTER 1 OVERVIEW

Black henbane can be either annual or biennial. I investigated which life cycle is found in four introduced western North American populations. Plants were grown in a greenhouse common garden until half were vernalized by exposure to natural winter temperatures, while the other half remained in the greenhouse above 20°C, with 16 hours of light and 8 hours of dark. In total the plants were monitored 313 days following germination. I measured whether plants bolted, the time it took for bolting to commence, and the size at bolting. All vernalized plants bolted after 117 days of active growth (within 26 days of the end of the vernalization treatment) while only 26% of the non-vernalized plants bolted after an average of 278 days of active growth. Vernalized plants bolted at a smaller size than the non-vernalized plants that bolted (28 vs. 41 leaves on average). In the non-vernalized plants, there was a strong relationship between time to bolting and size, but not so with the vernalized plants. My results indicate that introduced black henbane plants are biennial, and that vernalization is more critical to bolting and flowering than reaching a certain size. Nonetheless, the fact that non-vernalized plants were capable of bolting if grown long enough suggests that vernalization is not the only cue that can trigger reproduction in introduced populations.

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MANAGEMENT IMPLICATIONS

Knowledge of the life cycle of an introduced plant is a fundamental component of its successful management. Black henbane (*Hyoscyamus niger*) is a state-listed noxious weed that is toxic to both livestock and humans. There is surprisingly little information on the introduced populations in North America. I explored whether introduced populations follow an annual life cycle, biennial life cycle, or both. I first collected seeds from several naturalized populations. The seeds were germinated and the resulting plants were grown in a common environment in Fort Collins, Colorado. I then subjected half of the plants to a winter cold treatment (vernalization), while the other half remained in the greenhouse. All plants sampled appeared to be biennials, with cold being required for timely flowering. This has important implications for the potential of black henbane to spread: it is likely limited to areas that experience at least 10 weeks of cold (3°C-11°C) winter temperatures. Combining this information with this species being a poor competitor, requiring open space to thrive, we can infer that it will perform best in fairly open western North American environments with a cold winter. Additionally, given that it is biennial, where active management is necessary in the western United States, monitoring and managing populations over multiple years will likely be key to effective control. Finally, it is critical to guard against the introduction of annual henbane plants to North America. Currently, USDA-APHIS requires a permit to import any part of the *H. niger* plant or plant products into the U.S. Adhering to the current set of national regulations will help limit the range of naturalized black henbane populations.

INTRODUCTION

The life history strategy of an organism is an important factor in determining individual fitness and population growth rates (Stearns 1976), and in the case of invasive plants, helps determine whether a species establishes and becomes invasive in a new area (Sol et al. 2012; Sutherland 2004). The length of the life cycle of monocarpic plants is a key life-history trait. An annual life cycle may confer an advantage over biennial and perennial life cycles due to the shorter generation time (Baker 1965; Lewontin 1965; Pimm 1991). However, biennial plants often flower at a larger size and therefore typically produce more seed. Thus, there is potentially a trade-off between the cost of a longer generation time and the benefit of higher seed production (Klinkhamer and De Jong 1983; Van der Meijden et al. 1992; Wesselingh et al. 1993).

Both environmental and genetic factors can influence timing of reproduction in monocarpic plants (Johnson 2007; Reinartz 1984). The time to flowering and therefore generation time can be determined by plant size rather than age (Wesselingh et al. 1993). However, often exposure to a particular photoperiod (Parker et al. 1950) or to cold (e.g. vernalization, Bernier et al. 1981) determines a plant's ability to flower. Populations commonly vary, often along latitudinal clines, in the traits that determine timing of reproduction (e.g. pre-reproductive period, biomass, photoperiod, need for vernalization; Boudry et al. 2002; Quinn 1969; Reinartz 1984). Variation in these traits has frequently been shown to have a genetic basis (Law et al. 1977; Reinartz 1981). More recent studies have revealed a specific 'flowering locus', FLOWERING LOCUS C (FLC), in the model organism *Arabidopsis thaliana* that is regulated by vernalization and methylation (Finnegan et al. 2005; Michaels and Amasino 2000; Sheldon et al. 2000).

Here I explore two of the factors that influence life-cycle length (annual vs. biennial) of black henbane (*Hyoscyamus niger* L.; Solanaceae) in introduced North American populations. Specifically, I examine how plant size and vernalization influence flowering and time to flowering.

Study System

Hyoscyamus niger (commonly referred to as black henbane, or henbane) is an introduced toxic weed that typically grows in open and disturbed habitats. Henbane is a monocarpic plant with both annual and biennial forms in its native Eurasian range (Correns 1904; Schläppi 2011; Selleck 1964). The life history in introduced North American populations is currently unknown. Early research on life-cycle length in henbane suggests it is determined by a simple dominant allele (Correns 1904). Correns grew seed that had been collected from annual and biennial forms in a common field environment in Europe, and found that annuals always produced annuals and biennials always produced biennials. When he crossed the two forms, the F1 offspring were always biennial while the F2 offspring showed segregation, suggesting a single dominant locus determined whether a plant flowered in its first or second year. Further research confirmed that the annual life cycle is recessive (Correns 1904; Lang 1986; Schläppi 2011). Both annual and biennial henbane initiate flowering the first half of the summer season (i.e. they are long-day plants). Recent research on the genetic and physiological basis of flowering in *H. niger* suggests that there are two types of annuals, late flowering genotypes that benefit mildly from vernalization and early flowering genotypes that do not benefit from vernalization (Schläppi 2011). Under a constant temperature (22°C) and long-day photoperiod, Schläppi (2011) found that early-flowering annuals bolted after 30 to 35 days in pots and flowered at a size of 16 to 18

leaves, while late-flowering annuals bolted after approximately 60 days without vernalization and flowered with an average of 26 leaves. Non-vernalized biennial plants in Schläppi's study did not flower, even after one year. Additional studies on vernalization requirements of the biennial form of henbane indicate that at least 10 weeks of cold (i.e. 3°C-11°C) are required to trigger flowering (Diomaiuto-Bonnand et al. 1980; Melchers 1937). Thus, if introduced plants flower within 60 days, or shortly thereafter (in a greenhouse with a long-day photoperiod) without vernalization, we can infer that they are annuals, while if they do not flower in that time period, and respond to vernalization by flowering, we can infer they are biennials.

METHODS

The effect of vernalization on the life-cycle length of introduced black henbane plants was evaluated by experimentally imposing two temperature treatments (vernalized and non-vernalized) and subsequently measuring whether plants flowered or not, time to flowering, and size at flowering.

Seed was collected from four naturalized introduced populations in the fall of 2009. At each site I collected seed from 4-11 maternal plants (See Table 1.1 for sample sites and sizes). Following collection, seeds were removed from their capsules and stored in a refrigerator at 3.4°C until planting. Ten offspring were grown from each maternal plant. Seeds were sown in germination flats with Fafard potting media and placed on a mist bench with a misting regime of 15 seconds duration every 3 minutes for 9 hours per day over 12 weeks until enough seeds germinated. The median date of germination (here used as the general date of germination) was June 24, 2010, 6 weeks after sowing. Seven weeks after germination the seedlings were

transplanted to 1-gallon pots and kept in a greenhouse with a 16 hour day / 8 hour night photoperiod and 24.5°C day / 17.2°C night average temperatures.

Thirteen weeks after germination, on September 24, 2010, I recorded three measures of plant size: the total number of leaves larger than 2 cm, rosette diameter in two orthogonal axes, and the length and width of the largest leaf, and then initiated the treatments. Five of the 10 plants from each maternal line were randomly assigned to cold or warm temperature treatments. The plants in the cold treatment were moved outside to experience natural winter temperatures as the vernalization treatment, while the other half remained in the greenhouse. Vernalized plants were moved back into the greenhouse after 19 weeks outdoors, ensuring that plants experienced at least 10 weeks of cold temperatures (3°C-11°C) required for vernalization (Diomaiuto-Bonnand et al. 1980; Melchers 1937). The average daily temperature in Fort Collins, Colorado during the period used in my vernalization treatment was 3.89°C (National Weather Service data, accessed on wunderground.com). While outside, vernalized plants were buried in wood mulch and covered with straw. They experienced natural fall and winter weather conditions in Fort Collins, Colorado and received water by means of snow melt. Greenhouse plants were watered as needed, typically 3 times per week. In addition to the plants in the vernalization treatment experiencing colder temperatures than the non-vernalized plants, they also experienced a different light regime. Vernalized plants received low light overall (under mulch, in a lath house), and what light they received had the natural fall and winter photoperiods (short days, long nights). Non-vernalized plants received more light during the treatment period, with the greenhouse lights set to 16 hour day / 8 hour night.

Following the end of the treatment period all vernalized plants were brought back into the greenhouse. At this point the vernalized and non-vernalized henbane plants once again shared a

common garden environment. Days to bolting, days to flowering (with flowering defined as the first day that a flower was open enough to be pollinated), plant size (as described previously) and height (length of the tallest stem) at flowering were measured. Plant size was measured three times after the end of the vernalization treatment: on all plants one week after plants were recombined into the greenhouse (February 12), on all plants one week later (February 19) at which point the majority of the vernalized plants were bolting, and finally, individually as each plant started to bolt. These final measurements spanned 3 months from February 5, 2011 to May 3, 2011, at which point the experiment was terminated. Plants had grown for 313 days since germination, either entirely in the greenhouse or split into greenhouse (91 days), outdoors (132 days), and then greenhouse again (90 days).

Statistical Analyses

All statistical analyses were carried out with SAS® software version 9.3 (SAS Institute, Cary NC). I first evaluated whether or not the plants bolted, if the plants did bolt how long it took, and whether bolting depended on treatment or plant size. Because all vernalized plants bolted, there was no variation within that treatment (the separation problem; Albert and Anderson 1984), making it impossible to run a generalized linear mixed model (Proc. glimmix). Therefore, a Fisher's exact test was performed to evaluate the effects of the temperature treatment on the proportion of plants that bolted. Next, a mixed linear model (Proc. mixed) was used to evaluate the effect of rosette size before bolting on the time to bolting. Analyses included either the size in the fall or one of several different spring size measurements. Fall rosette size, measured as total number of leaves, produced the model with the lowest AIC value, and is presented in my results. Fall plant size, treatment, and a fall plant size by treatment interaction

were treated as fixed effects. Population, population by treatment interaction, and maternal plant nested within population were considered random effects. The significance of random effects was tested using likelihood ratio tests. I obtained -2 residual log likelihoods from running the model with and without the random effects, and the difference between those values provided a test statistic distributed as χ^2 with one degree of freedom (Littell et al. 1996). I evaluated size at bolting and I compared growth rates for vernalized and non-vernalized plants using models that included treatment as a fixed effect and population, population by treatment interaction, and maternal plant nested within population as random effects. For the non-vernalized plants, I also evaluated whether size in the fall or spring predicted whether or not plants bolted using a generalized linear mixed model with a binary distribution and a logit link. This comparison could not be done for vernalized plants, as all vernalized plants bolted (see Results and Discussion). Data were log transformed to meet the assumptions of ANOVA.

RESULTS

Vernalization strongly influenced the ability of plants to bolt and flower ($\chi_1^2 = 160.43$, $P < 0.0001$). In the 90-day period between the end of the vernalization treatment and the end of the experiment, 100% of vernalized plants bolted and flowered while only 26% of the 140 non-vernalized plants (n = 36) bolted (Figure 1.1). The time to bolting varied strongly by treatment (Table 1.2, Figures 1.1 and 1.2). On average, vernalized plants bolted 14 days and flowered 27 days after the cold treatment ended (105 and 118 days of active growth by which I mean total time since germination, excluding the vernalization treatment when the plants were largely dormant). All vernalized plants bolted within 26 days and flowered within 37 days after

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treatment (117 and 128 days of active growth). In contrast, for the non-vernalized plants that bolted, the average number of days to bolting was 278 days since germination (173 more days of active growth than the vernalized plants), and for those that flowered, the average number of days to flowering was 295 days since germination (177 more days of active growth than the vernalized plants).

The size of the rosette in the fall, before treatments were imposed, influenced time to bolting in the spring, and that influence differed in strength depending upon treatment (Table 1.2). Across both treatments, rosettes with more leaves in the fall bolted more quickly in the spring, but that pattern was stronger for non-vernalized plants (Figure 1.2). Even very small vernalized plants could bolt quickly, while only very large non-vernalized plants bolted in less than 26 days since plants were recombined into the greenhouse, or at 250 days of active growth (at which time all vernalized plants had bolted). Despite the important role of size in time to bolting of non-vernalized plants, size in the fall and spring did not predict whether or not those plants bolted (fall $F_{1,111} = 0.55$, $P = 0.4601$; spring first measure $F_{1,111} = 0.05$, $P = 0.8259$; spring second measure $F_{1,111} = 0.43$, $P = 0.5130$).

Models that included the other measurements of rosette size (results not shown) were consistent with the findings for fall rosette size data (Table 1.2), with larger plants always bolting earlier. Plant size at time of bolting differed significantly between treatments ($F_{1,3} = 117.88$, $P = 0.0017$). On average, vernalized rosettes bolted at a smaller size than their non-vernalized siblings (Figure 1.3). At bolting, non-vernalized rosettes reached an average size of 41 leaves while vernalized rosettes bolted with an average of 28 leaves. Rosette size for both treatments is measured as the total number of leaves at the time of bolting (i.e. fall rosette size was not added to the vernalized plants, which lost above ground biomass during the cold treatment).

Growth rates of vernalized and non-vernalized plants were calculated for the week between February 12 and February 19, the second week after the end of the treatment. After vernalization plants added leaves 1.7 times faster than the plants that had not been vernalized, though that difference was not significant ($F_{1,3} = 3.43$, $P = 0.1611$).

DISCUSSION

The fact that no plants flowered in the 90 days prior to the start of vernalization, and the rapid flowering of vernalized plants after their return to the greenhouse, clearly support the hypothesis that my study populations are composed of biennial henbane. However, the substantial percentage of plants that did eventually flower even without vernalization requires explanation. I propose and discuss 3 alternative hypotheses to explain the flowering of non-vernalized plants. 1. Late-flowering annuals, like those found by Schläppi (2011) are present in the introduced populations. In his study, 100% of late-flowering annuals bolted within 60 days without vernalization, while in mine, only 26% bolted, and that after a much longer 278 days. While an important hypothesis to consider, the long time to flower does not follow the life-cycle of a late-flowering annual, and thus this hypothesis is not supported by the current data. 2. Genetic variation weakening the requirement for vernalization may exist in the western United States that did not exist within Schläppi's samples. Schläppi (2011) was able to produce late-flowering winter annual *H. niger* plants from crossing biennial and annual accessions. It might be that hybridization between biennial and annual forms and subsequent backcrosses to biennials could lead to populations with weaker requirements for vernalization. No data are available currently to address this hypothesis. 3. Aspects of my experimental set-up weakened the

vernalization requirement. The two main differences other than cold between my vernalized and non-vernalized treatments are differences in the soil moisture content and in the light regime. As previously mentioned, vernalized plants received water via snow melt during the treatment. Non-vernalized plants were actively growing in the greenhouse and were watered as required which was typically three times per week. Therefore, non-vernalized plants in the greenhouse had higher soil moisture content than the vernalized plants for 19 weeks. There is no evidence from the literature that increased water availability can substitute for vernalization. The light regime in the greenhouse may have played a role, as photoinduction is a known replacement for vernalization in other systems (e.g. *Arabadopsis*, Bagnall 1993). Non-vernalized plants in my experiment received more light than the vernalized plants during the treatment period, with the greenhouse lights set to long days and short nights, the ideal photoperiod for flowering of black henbane (Downs and Thomas 1982; Lang 1986; Parker et al. 1950; Schläppi 2011). Thus, the long photoperiod and higher light intensity in the greenhouse relative to under mulch outside could have caused some non-vernalized biennial plants to flower. This hypothesis seems plausible, given the known mechanism from other systems, but additional data would help distinguish among the three.

My interpretation that introduced populations in Colorado, Wyoming, and Montana are dominated by biennial plants is supported by field data from more than 25 populations across those states. Germination took place in June, and marked plants did not flower until the following summer (C Fettig, unpublished data). An additional line of evidence comes from the current distribution of black henbane in North America; the USDA PLANTS Database reports this species to be found exclusively in areas that experience at least 10 weeks of cold winter temperatures, suggesting that vernalization is required in natural populations.

My results indicate that the plants from my sampled, introduced populations are strongly biennial. I believe this bodes well for land managers, as it likely limits the areas that henbane could invade. While my experiment demonstrated that vernalization is not absolutely required for bolting and flowering, it seems unlikely that seeds transported to climates without a winter cold period would successfully establish. In the greenhouse conditions, plants received sufficient resources (water, nutrients, light). However, in natural areas throughout much of the U.S. this would rarely be the case, with water being limiting at some period over the course of nearly 300 days in the west and southwest and other resources being limiting due to competition in the southeast. Indeed, henbane is found primarily in highly disturbed, open habitats (C Fettig, unpublished data) and is a poor competitor (LaFantasie and Enloe 2011). Therefore, it may be that the current distribution of black henbane in North America, which reflects the biennial life cycle I document here, represents a reasonably stable range as long as an annual form is not introduced.

Despite current efforts to prevent introductions of non-native species, introductions continue to occur (Cohen and Carlton 1998; Levine and D'Antonio 2003; Pysek et al. 2003). USDA-APHIS requires a "Permit to Import Plants and Plant Products" for any work with black henbane, along with a declaration that the seed is for research purposes only. However, black henbane is desired as an ornamental planting as well as for medicinal uses, and as such is cultivated in gardens still today. Given this, henbane seeds are readily available for purchase without a permit through internet vendors worldwide, despite the USDA-APHIS regulations. Furthermore, interstate movement of the plant is not regulated at the federal level. Given the commercial sale of seeds, eventual introduction of the annual form may be inevitable. However, Mitich (1992) reports that herbalists prefer the more productive and alkaloid-rich biennial form

of the plant providing some hope that the annual is not commonly planted. If introduction of the annual forms can be prevented, then *H. niger's* habitable range in North America may be restricted to northern climates.

Table 1.1. Locations of the *Hyoscyamus niger* seed collections that were used for this experiment, latitude and longitude of the sites based on GPS coordinates, and number of maternal lines that were collected from each population. Ten siblings were used from each maternal plant.

Location	Latitude	Longitude	Number of maternal lines
Parshall, Colorado	40 4.129	-106 15.438	5
Rock Springs, Wyoming	41 21.500	-109 16.165	4
Jackson, Wyoming	43 25.386	-110 46.524	11
Cascade, Montana	47 14.341	-111 51.927	8

Table 1.2. ANOVA results from the greenhouse experiment with *Hyoscyamus niger* evaluating the influence of fall plant size, treatment, and their interaction on the time to bolting in the spring. There was a significant interaction between rosette size (measured as number of leaves) in the fall and treatment. Fall rosette size of vernalized plants did not have a strong effect on time to bolting in the spring. However, non-vernalized plants that were larger in the fall bolted earlier than smaller non-vernalized plants.

Fixed Effects	df[a]	F	P
Number of leaves in fall	1, 136	22.96	<0.0001
Treatment	1, 3	123.43	0.0016
Number of leaves in fall*Treatment	1, 136	5.16	0.0246
Random Effects	df[a]	Likelihood ratio	P
Population	1	0.5	0.2398
Population*Treatment	1	0.1	0.3759
Plant(Population)	1	0	0.5

[a] Abbreviation: df, degrees of freedom.
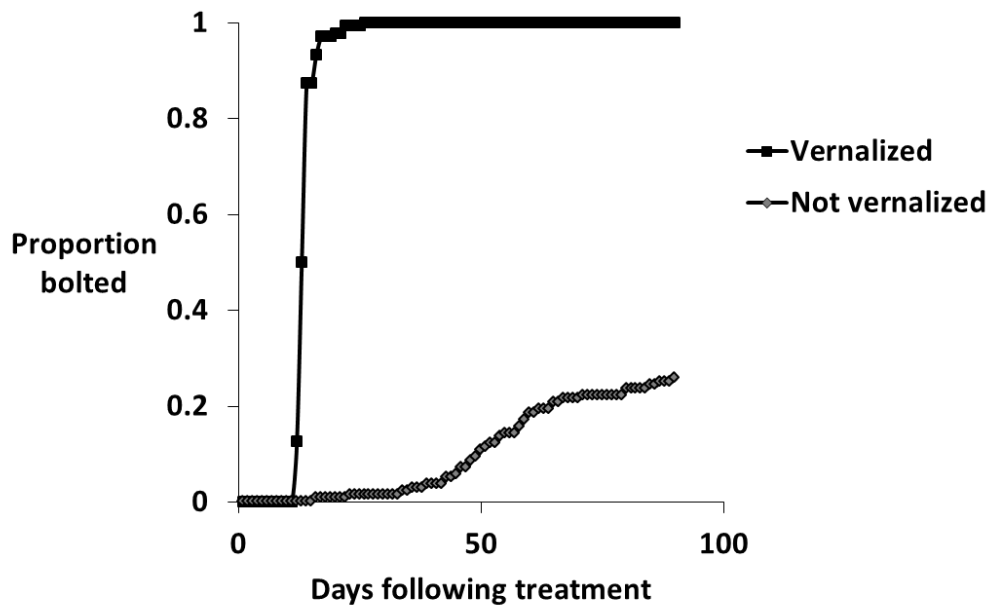
Figure 1.1. Cummulative proportion of plants from the two temperature treatments that bolted in the 90 days following the end of the vernalization treatment. One hundred percent of vernalized plants bolted within 26 days from the end of the cold treatment. Twenty-six percent of non-vernalized plants bolted within 90 days following treatment, or after 313 days of active growth.
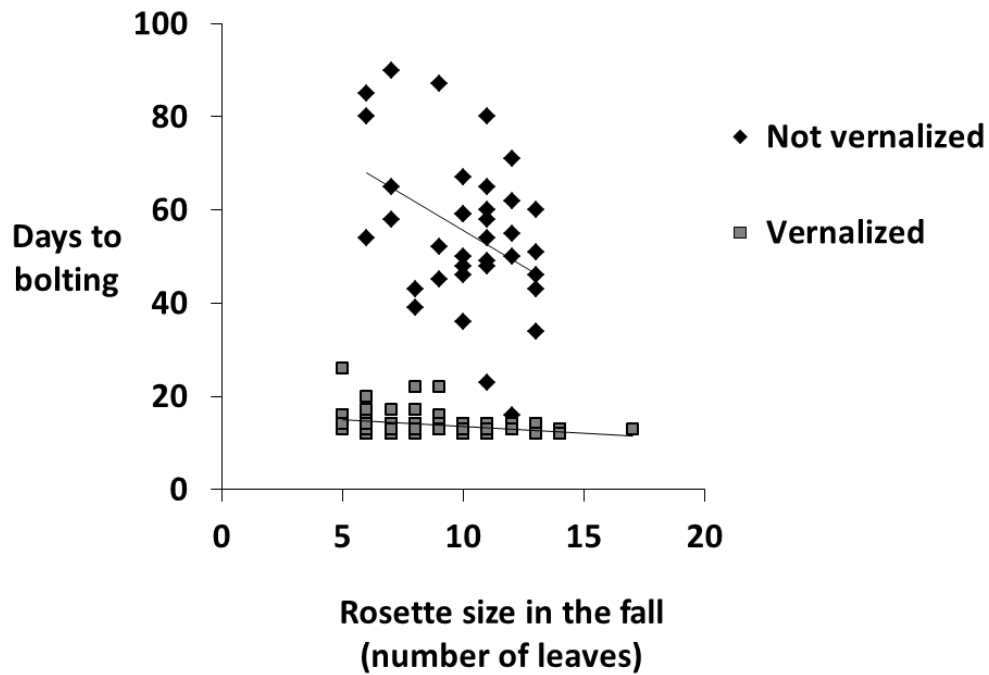
Figure 1.2. Number of days to bolting following the end of the vernalization treatment for plants in the two temperature treatments, based on fall rosette size. Non-vernalized plants that had more leaves in the fall bolted earlier in the spring than non-vernalized plants that had fewer leaves in the fall. Non-vernalized plants took longer to bolt than vernalized plants, in general. Number of leaves in fall*treatment is significant at $P = 0.0246$ (Table 1.2).
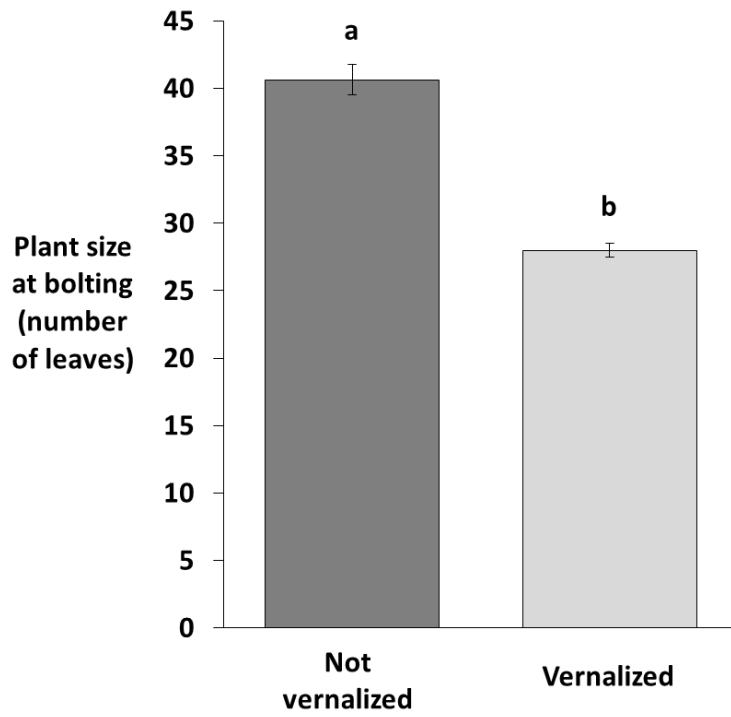
Figure 1.3. Mean number of leaves at bolting as a function of treatment. Data are means +/- 1 SE bars with different lowercase letters indicating that the means differ significantly at $P < 0.001$.

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CHAPTER 2: REPRODUCTIVE STRATEGY, PERFORMANCE, AND POPULATION

DYNAMICS OF THE INTRODUCED WEED BLACK HENBANE (*HYOSCYAMUS*

NIGER L.)

CHAPTER 2 OVERVIEW

Determining whether and how to manage an introduced species requires basic ecological and biological knowledge. If a decision is made to actively manage an invader, doing so efficiently and effectively is critical. Basic biological or ecological information can be key in designing effective and cost efficient management approaches. I used a greenhouse experiment and observational field studies of naturalized populations to study the modes of pollination, fecundity in the field, seed ecology, population dynamics, and demography of the introduced and toxic weed black henbane. I show that henbane is able to self-pollinate and that outcrossing does not increase number or size of seeds. Plants in the populations I sampled produce an estimated average of 25,300 ±4,004 seeds by the middle of the growing season. I found no difference in the viability of field collected seeds that were 1-4 years old. The number of flowering plants in a population is best predicted by the number of rosettes at that location in July of the previous year. The probability of rosettes surviving over the winter to reach reproductive maturity increases with precipitation and growing degree days. Total population sizes fluctuate dramatically between years. Henbane populations are ephemeral, but with large seed banks, large population outbreaks are possible if conditions are right. Given that this weed is toxic to livestock and humans, it is important to identify infestations and manage populations.

INTRODUCTION

Biological invasions are one of the top threats to biodiversity and global environmental sustainability (Mack and D'Antonio 1998; Pimentel et al. 2000). Worldwide, approximately eighty percent of all threatened and endangered species are on the protected list because of the influence and effects of non-native species (Wilcove et al. 1998). In the United States alone, the cost of managing invasive species and direct revenue loss due to invasion add up to a staggering $120 billion per year (Pimentel et al. 2005). Not all introduced species have extreme impacts in their new range, and thus not all require management. To determine both whether and how to manage these species requires basic ecological and biological knowledge.

If a decision is made to actively manage an invader, doing so efficiently and effectively is critical. Basic biological or ecological information can be key in designing effective and cost efficient management approaches. For example, successful eradication of the giant African snail (*Achatina fulica*) from Florida was achieved for the relatively low cost of $1 million (Capinera 2014). Simberloff (2003) argues that an important biological factor was essential to this eradication effort: that the snail is self-incompatible. This made eradication feasible, as the population had only to be reduced to the point where individuals had difficulty finding mates. Another example of the role of basic ecological information in successful management comes from the eradication of kochia (*Bassia scoparia*) from more than 3,200 hectares of western Australia (Randall 2001). In this case, the knowledge that kochia has a limited seed bank of less than three years (Zorner et al. 1984) was instrumental in planning the eradication process: sites had to be managed for a minimum of three years after the last seeds had set. In each of these examples, it was important to understand fundamental aspects of the biology of the system.

Whether trying to determine the level of threat that an introduced species poses or working toward eradication of a known, problematic invader, basic ecological information is necessary. However, even for some species introduced centuries ago, basic information is lacking. One such species is the introduced weed black henbane (*Hyoscyamus niger* L., Solanaceae).

Black henbane was introduced in the early 1600's to North America from Eurasia. It is toxic to the point of causing livestock and human poisoning and death (Cooper and Johnson 1984; Couch 1937; Kurkcuoglu 1970; Lindequist 1993; Long 1924; Spoerke et al. 1987). It is widespread across multiple western U.S. states, and is successful at colonizing disturbed habitats. Despite its toxicity, remarkably little is known about its ecology (Selleck 1964). In the western United States control of henbane populations is managed by weed supervisors at the state and county levels. Plants are managed in pasture lands and hay fields due to the plant's toxicity and threat to livestock, but efforts are also made to manage populations found elsewhere (e.g. public lands, disturbed areas, and roadsides). More information about the biology of this introduced species will provide insight into the ecology of its invasion, will help determine whether management efforts are warranted, and will provide the foundation upon which sound management approaches can be developed.

To understand the ecology of introduced populations of black henbane I address the following five topics and motivating sets of questions using a combination of experimental and observational studies.

1. Reproduction: Is black henbane capable of self-pollination? Does it benefit from outcrossing with plants from other populations?

2. Field performance: Are there differences in plant size by location? Does climate or

location influence differences in plant size? How much seed do plants in introduced

populations produce? Are there key climatological variables driving seed production?

3. Seed ecology: How large are soil seed banks? Do seeds remain viable multiple years?

4. Population dynamics: How large are introduced populations? Are there consistent trends
    in population size (i.e. growth or decline)?

5. Demography: When does mortality occur between rosette and adult? What proportion of
    rosettes reach adulthood? Is survival probability shaped by the immediate surroundings
    of a rosette?

By answering the above questions, my overarching goals are to understand basic

biological and ecological characteristics of black henbane in the introduced range, to lay the

groundwork for additional ecological and evolutionary research on this species, and to help guide

sound management practices.

Study System

Black henbane was introduced to eastern North America in the 1600's and is now found

in 27 northern states and throughout much of Canada (USDA PLANTS database). As of 2015,

henbane is a state listed noxious weed in California, Colorado, Idaho, Nevada, New Mexico, and

Washington. County weed supervisors in Wyoming are petitioning for henbane to be placed on

their state noxious weed list as well. Henbane is also listed on the Bureau of Land Management

National List of Invasive Weed Species of Concern.

It was initially introduced to North America by early settlers as an ornamental plant and

medicinal herb. A high concentration of scopolamine, hyoscyamine and other tropane alkaloids

made black henbane an effective treatment for toothaches, asthma, rheumatism, and stomach

pains, and it was also used as a general anesthetic in early medicine around the world (Biswas 1956; Husain 1979; Ratsch 1995; Rowell 1978). However, those same compounds, which are found in all parts of the plant, make it highly poisonous. Human poisoning and death following henbane consumption are common. For example, 31 cases of henbane poisoning in children were reported in Turkey over the course of three years (Kurkcuoglu 1970). However, the total number of henbane poisonings in that region was thought to be much greater, because the vast majority of the poisonings occurred in remote villages and children die on the journey to the nearest hospital (Kurkcuoglu 1970). Spoerke et al. (1987) reported the accidental ingestion and poisoning of two people in Montana, USA, when henbane growing on their property was mistaken for parsnip. Symptoms included vomiting and disorientation in the 11 hours following ingestion, but ultimately both subjects recovered (Spoerke et al. 1987). A number of other studies have also reported instances of henbane ingestion and poisoning (Beasley 1999; Doneray et al. 2007; Knight and Walter 2003; Long 1924; Sands and Sands 1976), or focused on the toxicity of the plant (Häkkinen et al. 2005; Lindequist 1993; Ma et al. 1999).

Little ecological information on black henbane has been published to date. Black henbane is a monocarpic plant with both annual and biennial forms in the native range (Correns 1904; Selleck 1964). It is the biennial form that composes populations in North America (Fettig and Hufbauer 2014). Biennial plants grow as a rosette during the first growing season. After experiencing cold winter conditions the plants bolt, flower, and set seed the following growing season. Black henbane has been reported to grow between 0.3 and 0.9 meters tall and produce tens of thousands of seeds per plant (Whitson et al. 2004). Seeds are scattered locally via gravity. It has not been reported how long seeds are viable in the seed bank. Plants typically grow in disturbed areas and are poor competitors (LaFantasie and Enloe 2011).

METHODS

Reproduction

To evaluate the degree to which introduced henbane is self-compatible, and whether inter-population outcrossing improves seed set or germination, I experimentally manipulated pollination in the greenhouse, conducting two experiments. In the first, I simply evaluated whether self-pollination is possible using two treatments, self-pollination with no manipulation and self-pollination by hand. In the second experiment I evaluated the effects on seed set of three treatments: a cross with a sibling, a cross with an individual from the same population, and a cross with an individual from a different population. For each cross, a vented polyethylene bag was secured over newly opened flowers to reduce the possibility of pollen contamination. For the first experiment in which plants were self-pollinated, anthers were left intact, while for each of the outcrossed treatments, anthers were removed prior to opening, in an effort to ensure that successful pollination was due to my experimental manipulations rather than self-fertilization.

Following my controlled crosses, the seed capsules were harvested once they were fully brown and mature on the stalk. I collected the seeds from each capsule, weighed them, counted a subsample, and stored them in a refrigerator at 3.4°C. To evaluate germination success, 10 capsules from each cross were randomly selected (5 capsules from maternal plants from Colorado and 5 capsules from maternal plants from Montana) for the experiment. I placed 2 replicates of 50 seeds per capsule on moist filter paper in Petri dishes. The dishes were kept in a germination chamber set to a 14 hour day / 10 hour night photoperiod and at an average temperature of 20°C. I ended the experiment after 47 days, at which point germination rate across all dishes had greatly decreased and 3 days had passed with no germination at all.

All statistical procedures in this publication were carried out using SAS® software version 9.3 (SAS Institute, Cary NC). I used mixed linear models as implemented in the SAS mixed procedure (Proc. mixed) to evaluate the difference in seed production, seed weight, and germination rates between naturally selfed and selfed by hand crosses, and between different crosses with different levels of outcrossing. Analyses included elevation of the site from which seeds were collected for the plants in the experiment, the type of cross, and the identity of the sire which were treated as fixed effects.

Field performance

I contacted county weed supervisors across three western states to locate populations that were not under active control to evaluate characteristics of these populations. I visited 33 populations of introduced black henbane in Colorado, Wyoming, and Montana in the summers of 2009, 2010, 2011, and 2012. Additionally, I was able to obtain data from one population in the native range, in France in 2010. Henbane populations in the native range may be ephemeral, as visits were made to 23 sites across southern France known have henbane within the previous 3 years, and only one site had a living population. Populations in the introduced range were revisited each year when possible. In each population, I measured plant performance of between 6 - 60 individuals. For each plant I recorded whether the plant was a rosette or bolting plant. For rosettes, I measured plant size from two perpendicular measurements from leaf tip to leaf tip and calculated rosette area using the formula for an ellipse. For bolting plants, I measured height, number of branches containing seed capsules, and the total number of developed/developing seed capsules. I selected plants for measurement from transect(s) that were 25 meters long and 2 meters wide through the most dense area of the population and sampled the plant closest to the

meter tape every 1 meter, for the length of the transect. If a population was large enough to hold two transects, then data were recorded from both. Climate data for each site over the duration of the study was acquired from National Weather Service data (Weather Underground, Inc.). Climate data for each site includes the total precipitation during May–September as well as for the previous year, mean temperature during May–September, mean temperature during the previous year's winter November – April, and total growing degree days each year as well as for the previous year. Multiple populations shared many of the weather stations as their closest available site. Therefore populations in my analyses were sorted by weather station and AIC model selection was performed prior to running the models described below.

I evaluated the relationship between plant height and the number of seed capsules produced per plant by the end of July using linear regression. Seed capsule data were log transformed to meet the assumptions of the model. To put the seed capsule data from North American populations into perspective, I compared them to seed capsule counts from two different locations, an introduced population in Britain (from Salisbury 1942) and the native population I sampled in France. The data from these other locations is limited, but I am able to make a coarse comparison using the 95% confidence intervals around the mean seed capsule production of North American plants, British plants, and French plants.

To evaluate the effect of climate on seed production, I first gathered data for each site from the nearest weather station with official National Weather Service data (Weather Underground, Inc.). Because many climate variables are correlated, I used AIC model selection to consider 7 different location and climate variables and their interactions in 9 different statistical models with seed capsule production and plant height as response variables (See appendix, Tables 4.1-4.4). In both of these models, weather station and population nested within

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weather station were treated as random effects. Both seed capsule data and plant height data were natural log transformed to meet the assumptions of the models.

Seed ecology

I surveyed the size of soil seed banks in three introduced populations by collecting soil samples from two henbane populations in southern Wyoming (Arlington and Rock Springs-2, see Table 2.1) and one population in Colorado (Parshall-3). Soil samples were collected in late October 2011 so the samples likely contained seed deposited from plants that matured in the 2011 growing season in addition to seed from previous seasons. I laid out a 20-meter transect through the densest portion of the henbane population and collected twenty cylindrical soil core samples that were 6.4 cm in diameter and 3.8 cm deep 1 meter apart from each other along the transect. I used fine sieves to separate materials in the samples based on size. Large rocks and fine dirt was discarded and the remaining sample was mixed in a beaker with water. I then poured the floating organic matter over coffee filters and allowed the processed organic matter from the soil samples to dry. Henbane seeds were identified using a dissection microscope, removed from the soil samples, and counted.

As a corollary to the seed bank question, I performed a germination experiment in a growth chamber at Colorado State University with seeds collected from plants in the field in an effort to determine if seeds remain viable multiple years. I used seed collected from 4 Colorado populations, 3 Wyoming populations, and 2 Montana populations in this experiment. Seeds were collected in the fall of 2008, 2009, 2010, and 2011 and stored at 2°C until they were used in this experiment in November 2012. Fifty seeds from mixed maternal sources but collected from the same population were placed on moist filter paper in plastic Petri dishes. Filter paper was

watered twice daily to maintain moist conditions. All seeds were kept in a germination chamber set to a 14/10 hour day/night photoperiod and 20°C temperature. As seeds germinated each day they were recorded and removed from the dish. I ended the experiment after 22 days, at which point germination rate across all dishes had greatly decreased and 3 days had passed with no germination at all.

I used a mixed linear model (Proc. mixed) to evaluate the difference in germination rates of seeds collected from plants in different years. Analysis included the collection year and the total growing degree days at the site during May 1 – September 30 of the collection year as fixed effects with state treated as a random effect. Germination rates were log transformed to meet the assumptions of the model.

Population dynamics

To determine the average size and variation of the size of introduced populations as well as trends in population growth and/or decline over multiple growing seasons I visited naturalized populations of henbane. For this effort, it was important that sites I surveyed were not treated with herbicides or other weed management practices. Although control efforts for this species are active and ongoing, I was able to sample 15 sites in Montana, Wyoming, and Colorado in July of 2009, 2010, 2011, and 2012. At each site, I counted the number of rosettes, the number of adult plants, and the total number of plants at each site.

I used repeated measures analysis with mixed linear models (Proc. mixed) to determine the factors that influence population size through time. My models included the previous year's population size as a fixed effect. Population size data were log (N+1) transformed to meet the assumptions of the model. I included a number of climatic variables and population attributes in

the analyses in an effort to predict population size from one year to the next. These variables included site elevation, yearly total precipitation at the site May–September as well as the total precipitation during the previous year, yearly mean temperature at the site May –September, yearly number of growing degree days May – September, the total number of plants in the population the previous year, the yearly number of rosettes present in the population as well as the previous year, and the yearly number of flowering plants present in the population as well as the previous year.

Demography

To determine the timing and frequency of plant mortality in introduced populations, I marked and tracked plants throughout their life cycle at 2 field sites. I visited sites located in Colorado (Parshall-3) and Wyoming (Arlington) in late May, mid-July, and late September of 2011, and late June 2012. Plants at these sites were not sprayed with herbicides or managed by land owners or county weed managers over the course of data collection. Two 1 by 9.7 meter transects at each site were marked with stakes and the boundaries of 60, 30.5 by 48 centimeter quadrants within those transects marked with nails. Uniquely numbered metal tags were nailed into the ground at the base of rosettes to mark all henbane rosettes within each transect. New rosettes were tagged as they were found in late May and mid-July of 2011 at both sites. I measured the area of each rosette as well as the length and width of the largest rosette leaf at my Colorado site in July 2011. I recorded total number of rosette leaves, an herbivory rating using the scale presented in Lewis et al. 2006, I measured vegetation composition and competition using 2 Daubenmire frames placed on opposite sides of the rosette and estimated percent cover of forbs, grasses, rocks, litter, bare ground, and other henbane plants for each rosette at both sites

in late September 2011. My final data collection at both sites was in June of 2012. At this point all plants were either bolting or dead. At this point I measured the height of all flowering plants, the number of branches, the total number of seed capsules, and an herbivory score of each adult plant. My count of seed capsules is not the total seed produced by each plant (because I visited in June rather than the fall), but my counts give a good comparison of plant size and maturity between the two henbane populations.

I used mixed linear models (Proc. mixed) to evaluate the effect of climate and first year plant characteristics on mature plant size (e.g. plant height and number of branches) and reproduction. I treated September 2011 rosette size and the interaction between mean temperature in 2011 and total precipitation in 2011 as fixed effects and population as a random effect. June 2012 plant height data were square root transformed to meet the assumptions of the model. Next, I used a generalized linear mixed model (Proc. glimmix) with binary distribution to assess the effect of first year plant characteristics, insect herbivory, and bare ground (i.e. competition) on plant overwintering survival. Analyses included population, rosette size in September 2011, the level of above-ground herbivory as measured in September, and the amount of bare ground around each rosette which were all treated as fixed effects.

RESULTS

Reproduction

The greenhouse pollination experiment showed that self-pollination can produce viable seeds. Hand-pollination improved seed production in selfed flowers, with hand-pollinated flowers producing, on average, a little more than twice as many seeds than flowers that were

bagged, but not pollinated by hand ($F_{1,29} = 10.11$, $P=0.0035$). Thus, introduced henbane has the ability to self, and outcrossing is not obligate.

Surprisingly, henbane does not benefit from outcrossing with plants from other populations, with comparable amounts of seed produced when plants were crossed with siblings, other plants within the same populations, or plants from different populations ($F_{1,4} = 0.12$, $P=0.7507$, Figure 2.1). There was also no significant difference in mass of individual seeds ($F_{1,47} = 0.37$, $P=0.5438$) between crosses. Therefore the similar results across cross types reflect not only the overall seed mass produced but also the number of seeds produced.

The crosses produced substantial numbers of seeds, comparable to the number of seeds per capsule produced by plants in the field. A random collection of 48 mature, unopened seed capsules from 2 different field populations yielded an average of 219.95 ±10.07 mg of seeds per capsule. My selfed crosses in the greenhouse produced an average of 187.87 ±14.90 mg of seeds per capsule (85.4% of the average yield of field plants) while my outcrossed plants in the greenhouse produced an average of 207.48 ±0.09 mg of seeds per capsule (94.3% of the average yield of field plants).

The mass of the seeds produced was linked to germination rates such that crosses that produced lower total seed mass had lower germination rates ($F_{1,15} = 19.68$, $P = 0.0005$, Figure 2.2). In general, though, germination rates were high: between 76.6 and 97.6 percent germination (Figure 2.2). Elevation from which the maternal plant was collected ($F_{1,15} = 0.07$, $P = 0.7954$), the cross performed ($F_{1,15} = 1.54$, $P = 0.2338$), and the sire ($F_{29,15} = 1.23$, $P = 0.3436$) did not affect germination rates.

Field Performance

On average across all populations, an individual plant produces 115 ±18.2 (mean ± 95% CI) seed capsules (estimated to be 25,300 ±4,004 seeds) by the end of July. This contrasts with data from 1936 and 1939 in introduced British populations (Salisbury 1942). There, plants produced an average of only 19 ±3.5 seed capsules. In southern France (the native range) in 2010 one population of 17 plants had an average of 52 ±19.6 seed capsules per plant. None of these confidence intervals overlap, establishing that my North American plants in the introduced range produce significantly more seed capsules than not only the population in France (native range), but also plants in Britain 80 years ago.

My field data showed that plant height is a good predictor of the number of seed capsules ($R^2 = 0.61$, $F_{1,523} = 787.19$, $P < 0.0001$, Figure 2.3). Model selection revealed precipitation and growing degree days to be most influential on seed capsule production and site elevation, precipitation and growing degree days to be most influential on plant height (See appendix, Table 4.1). The best model for seed capsule production included total number of growing degree days May 1 – September 30 and total precipitation May 1 – September 30 as fixed effects (See appendix, Table 4.2). The best model for plant height included total number of growing degree days May 1 – September 30, the previous year's total precipitation May 1 – September 30, the previous year's total growing degree days May 1 – September 30, and site elevation as fixed effects (See appendix, Tables 4.3 and 4.4).

Higher precipitation during the second summer, when seeds are formed, increased seed output ($F_{1,496} = 4.56$, $P = 0.0333$). Plants also have higher yield ($F_{1,496} = 16.11$, $P < 0.0001$), but are smaller in stature ($F_{1,748} = 42.19$, $P < 0.0001$) when they experience a greater number of growing degree days during the second summer of growth.

Of the populations I studied, plants at higher elevations (and lower latitude) are shorter ($F_{1,31} = 37.47$, $P < 0.0001$). A higher number of growing degree days ($F_{1,748} = 5.05$, $P = 0.0249$) and more precipitation ($F_{1,748} = 16.82$, $P < 0.0001$) during the rosette stage also yield shorter second year, mature plants.

Seed ecology

I collected as many as 469 seeds from a single soil sample (122 cubic centimeters). At that density there could be 146,093 seeds per square meter. On average, across all 3 sites, I estimate 15,596 ±4406 seeds per square meter. The greatest average density of seeds at one site, 33,424 ±9140 seeds per square meter, was found at Arlington, Wyoming (see Table 2.1 for location). This is likely due to the high plant density at that site.

Germination success of field-collected seed did not differ by collection year ($F_{1,69} = 1.81$, $P = 0.1834$). Germination rates were 12.6 ±5.3%, 29.9 ±7.1%, 24.1 ±4.3, and 16.0 ±9.1% for seeds that were 1, 2, 3, or 4 years old, respectively.

Population dynamics

Surveys of population characteristics and size reveal that henbane populations in the survey area are found exclusively in highly disturbed sites, and even then, are relatively small. Interestingly, the size of invasive populations fluctuates dramatically from year to year. There is not a pattern of consistent population growth or decline, rather populations increased in size by up to 40 fold and decreased in size by up to 100 fold over the course of three growing seasons (Figure 2.4).

Of all the plant count, location, and climate factors that were included in my model the only climatic factor that had an influence on total population size was total precipitation: a population that experienced higher levels of precipitation one year was likely to have a larger total population size the following year ($F_{1,29} = 6.65$, $P = 0.0152$). Surprisingly, even the total population size one year did not predict the next year's total population size ($F_{1,29} = 0.60$, $P = 0.4453$), though the number of rosettes present in a population at the end of July was positively associated with the number of flowering plants in that population the following year ($F_{1,29} = 8.01$, $P = 0.0084$).

Demography

Plants that were tagged and monitored over the 2011/2012 growing season revealed both timing of germination and of mortality (Figure 2.5). While both germination and mortality span the entire length of the growing season, typically May – September, there were two main periods when rosette death occurred: July-September, when the plants were first year rosettes and over winter (Figure 2.6). The Wyoming site experienced the biggest loss (57%) in number of rosettes between July and September while the Colorado site had the biggest loss of rosettes (63%) over the winter. Overall, 18.4% of all tagged Colorado plants survived to reproduce while 30.8% of all tagged Wyoming plants survived to reproduce. Interestingly, smaller rosettes in September 2011 not only survived the winter, but became the taller plants in June 2012 ($F_{1,44} = 9.95$, $P = 0.0029$).

In addition to monitoring plant survival, I also evaluated the effects of climate variables on plant performance. Plants that experienced the 2011 growing season (May 1, 2011 – September 30, 2011) with a higher mean temperature and more precipitation (the Wyoming

plants) were taller (precip11*temp11, $F_{1,44} = 154.41$, $P < 0.0001$), had more branches ($F_{1,44} = 45.59$, $P < 0.0001$), and had more seed capsules ($F_{1,44} = 27.34$, $P < 0.0001$) when they were measured in June 2012.

Overwintering survival of rosettes differed significantly between populations ($F_{1,102} = 6.86$, $P = 0.0101$), but rosette area in September 2011 ($F_{1,102} = 0.30$, $P = 0.5872$), level of above ground herbivore damage ($F_{1,102} = 1.69$, $P = 0.1966$), and the amount of bare ground ($F_{1,102} = 0.17$, $P = 0.6847$) did not predict overwintering survival well. Within the individual populations none of the variables I tested predicted overwintering survival well, though there was a trend that rosettes that were surrounded by more bare ground in 2011 had a higher probability of survival (Figure 2.7, $F_{1,43} = 0.04$, $P = 0.8519$).

DISCUSSION

The questions of whether and how to manage an introduced species depends, at least in part, on population dynamics. If populations of a well-established invader (beyond the point at which eradication might be feasible) are growing and becoming more dense, then they might warrant management to reduce further spread as well as minimize ecological effects on local habitats. However, if populations are not consistently growing, then management efforts could perhaps be better focused elsewhere. Knowledge of the underlying factors that can drive plant population size fluctuations (e.g. reproductive strategy and capacity, seed bank capacity and seed longevity, plant demographics within populations) and how unmanaged populations persist over time need to be considered when creating management programs. An understanding of differences between native and introduced plants can help illuminate some of the reasons for

success of introduced populations. In black henbane's case, one important difference I found is the fact that introduced plants are self-compatible.

The family Solanaceae is largely self-incompatible, with the self-incompatibility mechanism (S-locus) being basal to the group, and approximately 90 million years old (Steinbachs and Holsinger 2002). Some solanaceous species retain the ancestral self-incompatibility mechanism (Igic et al. 2004; Miller et al. 2008; Richman and Kohn 2000) while many have transitioned from self-incompatible to self-compatible (Igic et al. 2004, Igic et al. 2006). The shift from self-incompatibility to self-compatibility is one of the most common evolutionary changes in angiosperms (Stebbins 1974). Self-incompatibility is often lost within a taxon and the transition is rarely, if ever reversed (Igic et al. 2008). Results from my pollination experiment provide evidence that introduced henbane is able to self, and is not an obligately outcrossing species. This is the first confirmation of self-compatibility in introduced henbane, and the only confirmation for the species in the published (English language) literature. Whether native populations of this species are able to self is unknown. It is conceivable that introduced populations of henbane may have evolved the ability to self over time, either prior to or following introduction and that self-compatibility may facilitate the spread of this species (Stebbins 1957, and see Baker 1967).

In a predominantly self-fertilizing population, selection against deleterious alleles will be strong and after such alleles are purged from a population, the potential advantages of outcrossing will be reduced. True to the models presented by Stebbins (1957) and Lande and Schemske (1985), henbane is a selfing, introduced species that does not show an advantage from outcrossing in this study. The lack of an outcrossing advantage shown in my pollination experiment could also be because introduced populations of henbane may already have high

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genetic diversity within populations. Alternatively, it is also possible that we do not see an advantage from outcrossing in my experiments because in this system, an advantage may not be expressed until the plant is older, sometime after seed production and germination.

It is important to note that the mass of seeds produced in my two greenhouse pollination experiments should not be compared. Anthers were left intact in my self-pollination experiment whereas anthers were removed in all of the treatments in my outcrossing experiment. In a separate experiment I found a significant effect of anther presence, whereby removing anthers actually increased seed set (data not shown).

My record of plants in naturalized populations producing 115 ±18.2 seed capsules, on average, contrasts with the data from Salisbury (1942) and observations collected in southern France in 2010. Though the native range data are scant, they are consistent with a pattern seen in many other invaders, introduced individuals and populations are larger (Abela-Hofbauerova and Munzbergova 2011; Blumenthal and Hufbauer 2007; Brown and Eckert 2005; Dlugosch and Parker 2008; Flory et al. 2011; Fukano and Yahara 2012; Harris et al. 2012; Hodgins and Rieseberg 2011; Huang et al. 2012; Keller and Taylor 2010; Lavergne and Molofsky 2007; Rogers and Siemann 2005; Wolfe et al. 2004).

My studies showed a significant effect of growing degree days leading to higher seed output of henbane plants. Higher moisture levels and temperatures lead to higher survival and fitness of henbane. As the climate changes and temperatures increase, we may see a change in henbane populations to be more dense and less ephemeral. Higher seed production in introduced plant species has been documented in other systems as a result of climate change (Redman et al. 2011; Smith et al. 2000; Wookey et al. 1993) and thus should be seriously considered in future research of black henbane.

Henbane seeds typically fall to the ground directly below the plant where they were produced. This creates soil seed banks that are rich with henbane seed in the top few inches of soil. Intuitively, the size of the seed bank increases with plant density. Seeds remain viable over multiple years with no obvious difference in germination rates between seeds that have matured in the last year as opposed to seeds that matured 4 years earlier. Other than chilling (storage of seeds in a refrigerator) I did not use any form of chemical germination promoter or scarification in my seed germination experiment. I did, however, notice a difference in germination rates when comparing my pollination germination results to my field collected seed germination results. Seeds produced from my pollination experiment had a higher overall germination rate (85%) while field collected seed had a much lower germination rate (20%). Seeds germinated in both experiments were stored for similar amounts of time at the same temperature and both germination experiments were carried out following the same protocol. Seeds were also collected off of plants in the greenhouse and from plants in the field at the same point of seed capsule maturity. I hypothesize that hand pollination produces seeds with higher germination rates when compared to naturally pollinated seeds in the field. Lower germination rates in field pollinated seeds may be attributed to a pollen-transmitted fungal pathogen or virus vectored by a pollinator, as has been observed with *Tobacco ringspot-virus* which is known to infect solanaceous plants (Yang Hamilton 1974). However, other studies that include the germination of henbane seeds report varying levels of success, between 0 and 80% germination, so it is possible that germination of henbane seeds is inherently variable (Cirak et al. 2004; Sharma et al. 2006).

The germination rates observed in my trial with field collected seeds are reasonably comparable to natural germination rates that would occur in the field (Radosevich et al. 1997). I note from field observations that germination begins as early as May and may occur as late as

October. While we know that there are many seeds in the seed bank and seeds from multiple years germinate, populations are not consistently large in size. Thus, the size of soil seed banks and seed germination rates are not likely to limit population size after initial establishment.

Populations of introduced henbane vary in size from one location to another, and from year to year. If black henbane were aggressively invasive in this region, then we would expect populations to increase in size, but they are not. I should note that my selection of a limited number of unmanaged sites is not a random sample, and so the populations used in this study might be smaller populations than those populations that are managed at other locations. The dynamic fluctuations in population size of the locations I studied could be due to climatic factors. In both my field survey of population size changes over time and my demography study, higher levels of total precipitation at a site led to high yielding mature plants as well as larger overall population sizes. Population sizes fluctuate dramatically between years and cannot be predicted confidently from one year to the next because future precipitation levels, the size of the soil seed bank, and availability of open spaces for germination are typically unknown.

In my demography study both populations exhibited great fluctuation in total size over time as well as considerable plant mortality in the middle of the growing season. The amount of total plant mortality over the entire growing season that I recorded at my Wyoming site (69.2%) and my Colorado site (81.6%) is similar to that found in other overwintering biennial plant species (*Verbascum thapsus* Gross 1981 [79%, 30%, 57%, 38%]; *Picris hieracioides* Klemow and Raynal 1985 [55-70% & 80%]; *Lysimachia rubida* Suzuki et al. 2003 [94% & 98% mortality]).

Water availability and competition with other plants are probably the most limiting factors to henbane population size and growth. Selleck (1964) found that seeding an already

infested area of henbane with crested wheatgrass or bromegrass resulted in complete control of the henbane. He also found, however, that black henbane infestations maintained their vigor in areas with greater water availability (Selleck 1964). More recently LaFantasie and Enloe (2011) presented henbane as a poor competitor in their experiment with 3 native grasses. LaFantasie and Enloe (2011) grew henbane with and without 3 species of native perennial grasses that are typically used in restoration projects. Total biomass of henbane was found to be as much as 99% lower when the plants were grown in the same pot as mature grasses. Competition with henbane had little effect on the species used as competitors (LaFantasie and Enloe 2011).

My data emphasize the importance of precipitation for henbane populations to be large and composed of high yielding plants. If water availability and competition are indeed limiting factors for henbane population success, then populations may be limited to areas that experience adequate rainfall.

Introduced henbane is able to self and plants produce copious seed. Seed banks are large and seeds remain viable for at least four years with no apparent decline in viability. Growing seasons with more growing degree days and ample precipitation encourage large population sizes, although population sizes fluctuate dramatically over time. When there is population decline, it may be due to the high number of rosettes that do not survive the winter. It is possible that introduced populations of henbane will naturally fluctuate and disappear (without the need for common weed management practices and herbicide application) when they are outcompeted by native or other introduced plant species. We now know that populations in the western United States are ephemeral, but the seed banks and potential for large population outbreaks are present. However, henbane is prolific and has been shown to cause serious threats to livestock and humans. Given that this weed is toxic, it is crucial to identify infestations and manage

populations. To do this, plants should be controlled prior to seed set. Where populations persist,

it is best to control henbane infestations with tillage and planting of competitor native grasses

and plants.

Table 2.1. Location and elevation of field observation and seed collection sites, GPS coordinates, and the years in which each site was visited.

State	County	Location	Elevation of site (m)	GPS Coordinates	Years visited
Introduced range					
Colorado	Grand	Granby	2373	40.1501, -105.9284	2009, 2010, 2011
		Kremmling	2402	40.1257, -106.3349	2009, 2010, 2011, 2012
		Parshall-1	2481	40.0002, -106.1309	2010, 2011
		Parshall-2	2367	40.1046, -106.0012	2009, 2010, 2011, 2012
		Parshall-3	2343	40.068, -106.2573	2009, 2010, 2011, 2012
		Parshall-4	2363	40.0349, -106.2382	2009, 2010, 2011, 2012
Montana	Beaverhead	Dillon-1	1581	45.173, -112.7026	2009, 2010, 2011, 2012
		Dillon-2	1737	44.9865, -112.9972	2009, 2010, 2011, 2012
		Dillon-3	1865	44.6976, -112.7098	2009, 2010, 2011, 2012
		Dillon-4	1986	45.168, -112.4303	2009, 2010, 2011, 2012
	Broadwater	Townsend-1	1395	46.2248, -111.3733	2010, 2011, 2012
		Townsend-2	1160	46.3355, -111.5086	2010
		Townsend-3	1429	46.252, -111.2929	2010, 2011, 2012
		Townsend-4	1222	46.3225, -111.5485	2009
		Townsend-5	1436	46.3179, -111.6332	2009, 2010, 2011, 2012
		Townsend-6	1270	46.0785, -111.578	2009
	Cascade	Belt	1228	47.2805, -110.8164	2009, 2010, 2011, 2012
		Cascade	1404	47.239, -111.8654	2009, 2010
		Great Falls	1031	47.4079, -111.3062	2010
	Park	Livingston-1	1369	45.6847, -110.5149	2009, 2010, 2011, 2012
		Livingston-2	1399	45.7041, -110.4115	2009, 2010, 2011, 2012
Wyoming	Carbon	Arlington	2356	41.6069, -106.2082	2009, 2010, 2011, 2012
		Rawlins-1	2241	41.7491, -106.4638	2009, 2010, 2011, 2012
		Rawlins-2	2180	41.7879, -106.4672	2009
	Park	Cody	1678	44.4832, -109.3459	2009
	Sweetwater	Rock Springs-1	2301	41.0566, -109.3376	2010, 2011, 2012
		Rock Springs-2	2301	41.372, -109.139	2010, 2012
		Rock Springs-3	2346	41.4515, -109.171	2009, 2010, 2011
		Rock Springs-4	2244	41.3583, -109.2694	2009, 2010, 2011, 2012
	Teton	Jackson Hole-1	1876	43.3893, -110.7347	2010, 2011, 2012
		Jackson Hole-2	1832	43.4249, -110.7757	2010, 2011

	Jackson Hole-3	1891	43.435, -110.7764	2010, 2012
	Jackson Hole-4	1874	43.4231, -110.7754	2009, 2010
Native range				
France	Causse-de-la-Selle	306	43.78177, 3.60095	2010
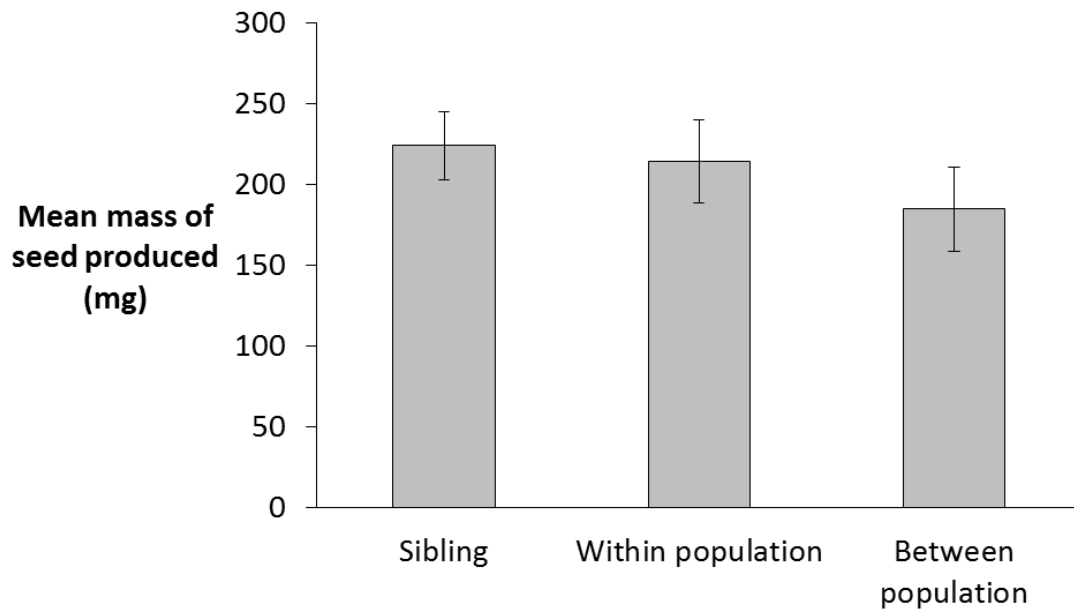
Figure 2.1. Mean mass of seed produced (mg) as a function of the breeding cross that was performed. Data are means +/- 1 SE bars. Differences between treatments are not significant ($F_{1,4}$ = 0.12, P=0.7507).
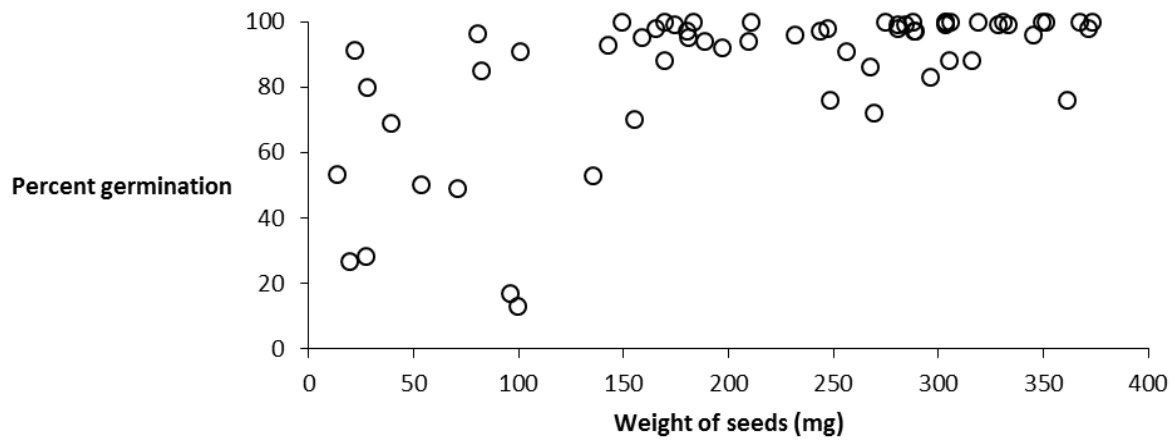
Figure 2.2. Percent germination of seeds produced in the pollination experiment. Crosses that produced greater mean total seed mass had higher germination rates overall.
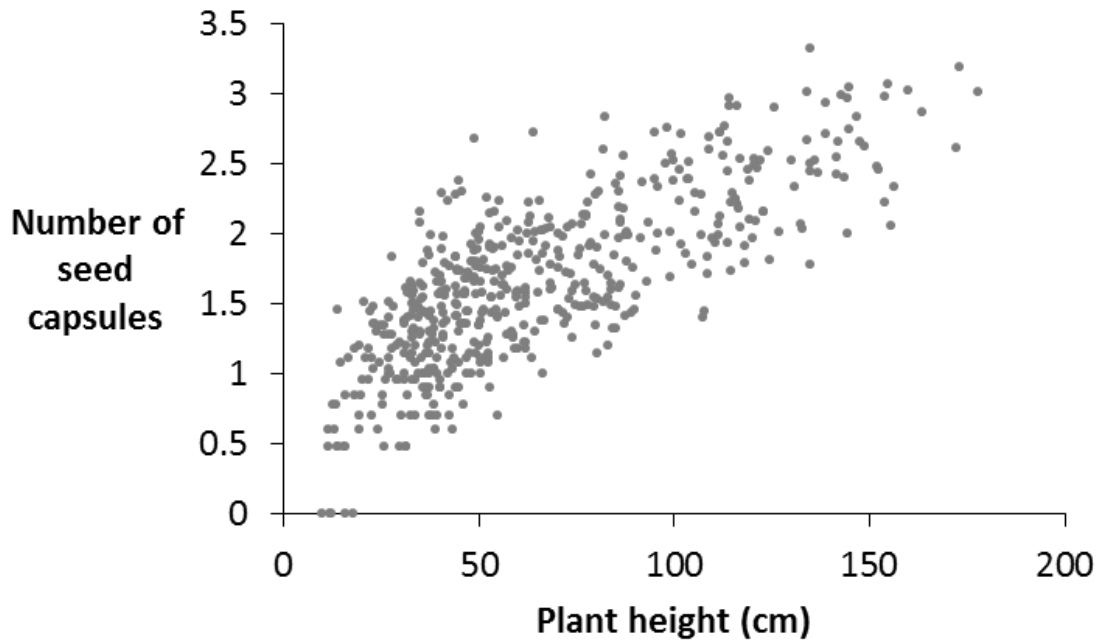
Figure 2.3. Relationship between plant height observed in naturalized populations in the field and the number of seed capsules present on each plant in July. Data have been log transformed for this figure.
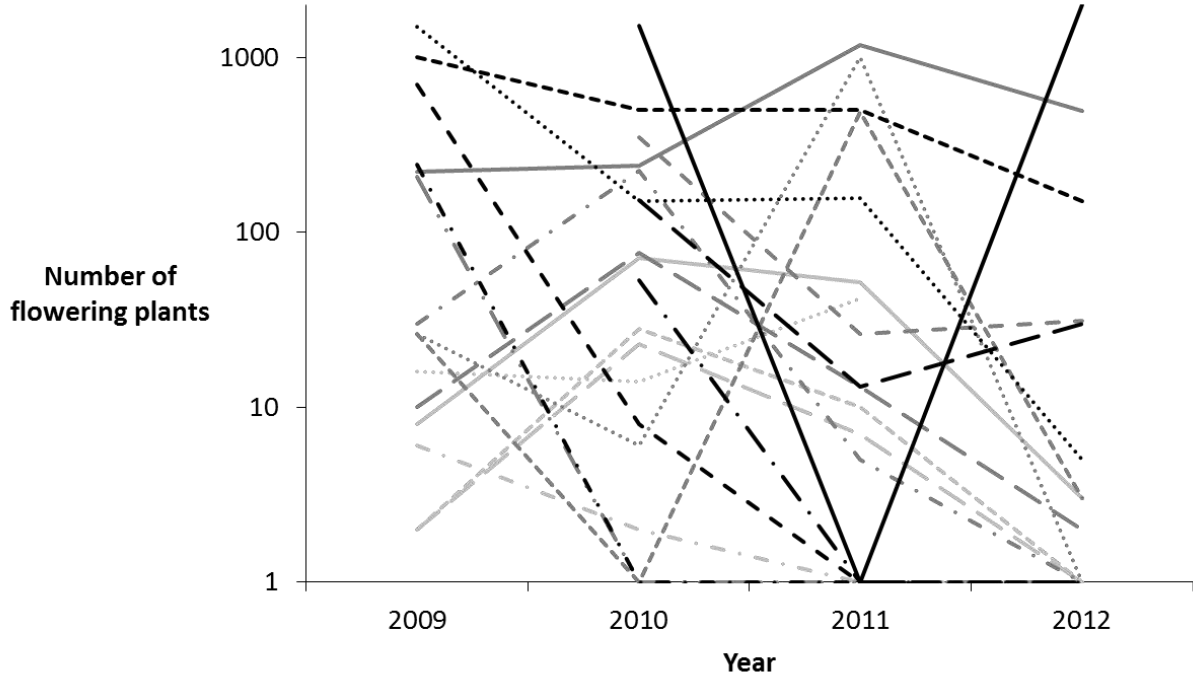
Figure 2.4. Data shown here, transformed on a log scale, show the larger pattern of population dynamics within and among my study sites in Colorado, Montana and Wyoming. There are dramatic fluctuations in population sizes over 3 growing seasons.
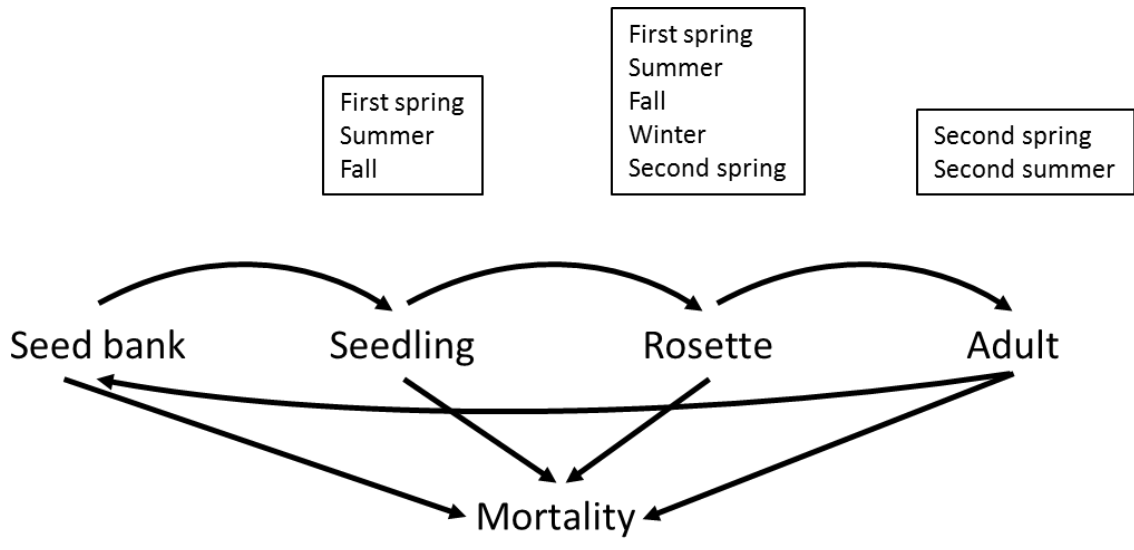
Figure 2.5. Schematic diagram of the life cycle of black henbane. Seasonal timing of each stage of plant growth is represented in the boxes above each growth stage.
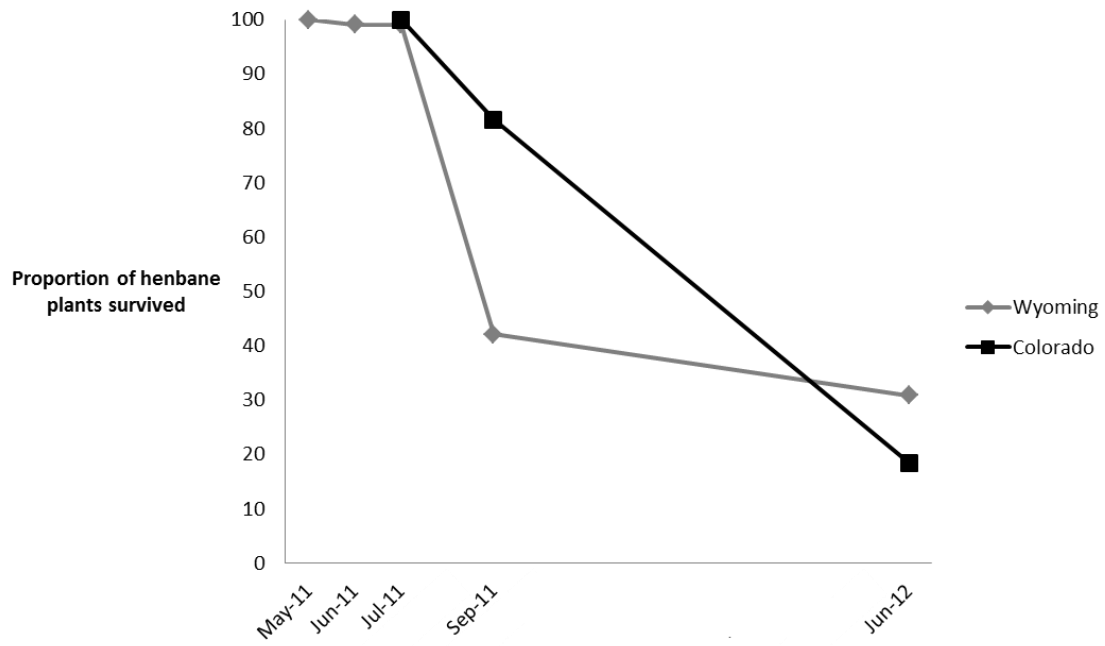
Figure 2.6. The proportion of henbane plants in my demography study that survived over time. Data are from two locations in Colorado and Wyoming.
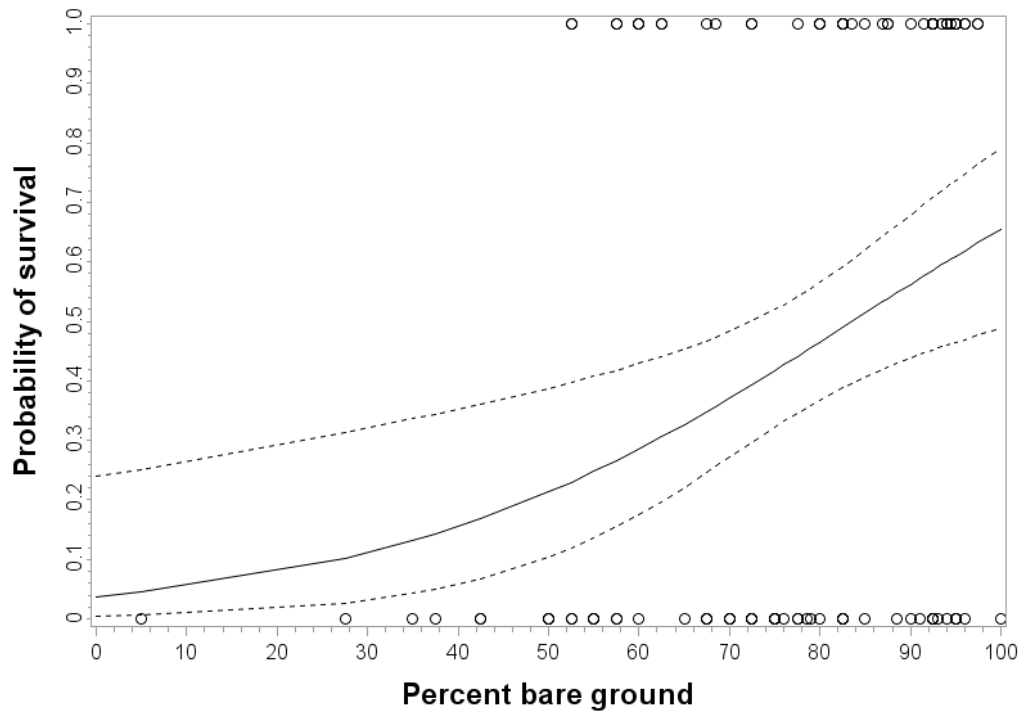
Figure 2.7. Percent bare ground surrounding each plant vs. the probability of the plant's survival. The solid line is the direct logistic regression and the dashed lines are 95% confidence intervals.

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CHAPTER 3: SIMILAR LEVELS OF RESISTANCE AND TOLERANCE IN NATIVE AND

INTRODUCED PLANTS OF COMMON MULLEIN (*VERBASCUM THAPSUS* L.)

CHAPTER 3 OVERVIEW

A number of hypotheses put forth to explain the success of invasive plants posit that changes in plant defense facilitate invasion success. Reviews of the literature show mixed support for changes in herbivore pressure and levels of resistance. There are few data available on the role of tolerance in plant invasions. Because a tradeoff in plant defense characteristics could potentially facilitate invasion, it is important to study both resistance and tolerance as mechanisms of defense in plant invasions. Here I measure resistance and tolerance of native and introduced *Verbascum thapsus* (common mullein) in a collection of eight different common garden experiments. A specialist herbivore (*Gymnetron tetrum*) and a generalist herbivore (*Trichoplusia ni*) did not show a preference between native and introduced plants in choice experiments, suggesting plants from both ranges have similar levels of defense to herbivory. The specialist weevil fed more on highly defended young leaves from introduced plants. *Trichoplusia ni* larvae fed more on older leaves. Tolerance to clipping did not differ between native and introduced plants. Indeed, there was no significant difference in the amount of above ground biomass re-growth or in the final below ground biomass between control plants and those that lost 60% of their aboveground biomass. These results suggest that, under the environmental conditions of my experiment, both introduced and native mullein are similarly defended and extremely tolerant. Comparative levels of defense to the native range and high tolerance may contribute to mullein's success in the introduced range.

INTRODUCTION

Two important hypotheses to explain what enables a species to become invasive are the enemy release hypothesis (Keane and Crawley 2002) and the evolution of increased competitive ability hypothesis (EICA, Blossey and Nötzold 1995). The enemy release hypothesis proposes that invasive species escape the top-down population regulation imposed by their native natural enemies (including herbivores, for plants). A consequence of enemy release for invasive plants is reduced selection for resistance against herbivores, and this has been hypothesized (EICA) to further facilitate invasion by freeing resources for growth (Blossey and Nötzold 1995). There is strong support for enemy release in a number of invasive plant populations (Adams et al. 2009; Keane and Crawley 2002; Liu and Stiling 2006; Mitchell and Power 2003; Wolfe 2002), and mixed support for EICA.

Several direct tests of EICA lend support to the hypothesis (Blumenthal and Hufbauer 2007; Leger and Rice 2003; Siemann and Rogers 2001; Wolfe et al. 2004). However, a number of studies show no difference in size or reproduction between native and exotic conspecifics (Maron et al. 2004; Thébaud and Simberloff 2001; van Kleunen and Schmid 2003; Willis et al. 2000), and one (Bossdorf et al. 2004a) provided evidence for the inverse: reduced competitive ability in invasive *Alliaria petiolata*. Reviews of the literature confirm this mixed support across multiple studies (Bossdorf et al. 2005; Hinz and Schwarzlaender 2004).

Müller-Schärer et al. (2004) further refined the EICA hypothesis by distinguishing between specialist and generalist herbivores. While introduced plants often gain generalist herbivores in their new range, it is typically the specialist herbivores that they escape. Effective defenses vary for specialists and generalists (Ali and Agrawal 2012) and levels of defense may vary not only among plants, but may also vary within a single plant (Traw and Feeny 2008; van

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Dam et al. 1995). The optimal defense theory posits that differences in risk of herbivory select for different parts of plants to be more or less defended, and in particular that nutrient rich young tissues should be more defended than older tissues (McKey 1974; Rhoades and Cates 1976). Research on optimal defense theory indeed demonstrates that younger, more valuable leaves are in general more highly defended than older leaves (Alba et al. 2012; Ohnmeiss and Baldwin 2000; van Dam et al. 1996; Zangerl and Rutledge 1996). When considering the larger picture of plant defense and plant-insect interactions, however, resistance against herbivory is not the only strategy plants use in coping with herbivores and their damage. Tolerance can also be an important mechanism of plant defense (Müller-Schärer et al. 2004; Strauss and Agrawal 1999).

Tolerance to herbivory is the ability of a plant to compensate for damage through re-growth or reproduction (Ashton and Lerdau 2008; Strauss and Agrawal 1999; Weis et al. 2000). Ecological theory predicts a tradeoff between resistance and tolerance to herbivory (Strauss and Agrawal 1999), and a number of studies confirmed this prediction (Fineblum and Rausher 1995; Leimu and Koricheva 2006; Mauricio et al. 1997). Currently, there are few data available on the role of tolerance in plant invasions, but one species, *Sapium sebiferum*, exhibits increased tolerance and decreased resistance to native insect herbivores (Zou et al. 2008a). Many invasive species grow faster (Siemann and Rogers 2003; van Kleunen et al. 2010) and larger than native conspecifics (Abela-Hofbauerova and Munzbergova 2011; Blumenthal and Hufbauer 2007; Brown and Eckert 2005; Dlugosch and Parker 2008; Flory et al. 2011; Fukano and Yahara 2012; Harris et al. 2012; Hodgins and Rieseberg 2011; Huang et al. 2012; Keller and Taylor 2010; Lavergne and Molofsky 2007; Rogers and Siemann 2005; Wolfe et al. 2004). The fact that tolerance is correlated with increased growth (Ashton and Lerdau 2008; Weis et al. 2000), suggests the possibility that plant invasions may be facilitated by a greater tolerance to damage

by generalist herbivores. If tradeoffs exist, this could be linked to decreases in resistance to herbivory. It is therefore important to examine both resistance and tolerance as mechanisms of defense in invasion ecology (Chun et al. 2010; Zas et al. 2011).

A small but growing body of literature compares tolerance or both tolerance and resistance between native and introduced plant populations (Abhilasha and Joshi 2009; Bossdorf et al. 2004b; Huang et al. 2010; Rogers and Siemann 2004; Rogers and Siemann 2005; Wang et al. 2011; Zou et al. 2008a; Zou et al. 2008b). Seven of these (Abhilasha and Joshi 2009; Huang et al. 2010; Rogers and Siemann 2004; Rogers and Siemann 2005; Wang et al. 2011; Zou et al. 2008a; Zou et al. 2008b) out of the eight studies found increased tolerance in introduced populations relative to native ones while one (Bossdorf et al. 2004b) found no difference in tolerance in introduced versus native populations. Five of the eight studies measured resistance in addition to tolerance (Abhilasha and Joshi 2009; Bossdorf et al. 2004b; Wang et al. 2011; Zou et al. 2008a; Zou et al. 2008b). One study found increased resistance in introduced plants (Abhilasha and Joshi 2009), one study found no difference between ranges (Bossdorf et al. 2004b), and three studies (Wang et al. 2011; Zou et al. 2008a; Zou et al. 2008b) found evidence for increased tolerance as well as reduced resistance in introduced populations relative to native ones.

Verbascum thapsus (common mullein, hereafter mullein) is an ideal system to compare levels of resistance and tolerance of herbivory as much comparative data are available on native and introduced populations. Plants from introduced populations grow faster and larger than those from native populations (Alba et al. 2011) and there are differences in allocation to defenses against generalist herbivores between different ages of leaf tissue of introduced plants (Alba et al. 2013). Alba et al. (2013) found support for the optimal defense theory with younger leaves

being better defended from a generalist herbivore (i.e. higher levels of iridoid glycosides as a chemical defense) than older leaves. Additionally, the magnitude of the difference in defense allocation is larger for introduced plants than native plants (Alba et al. 2013). Here I use mullein plants from a broad sampling of both native and introduced populations to determine if there are differences between plants from both ranges in resistance to a generalist and a specialist herbivore, if resistance levels to those herbivores vary with leaf age, and if there are differences between ranges in plant tolerance to simulated herbivory.

METHODS

Study system

Common mullein is native to Eurasia and was introduced to North America by early European settlers for use as a medicinal plant (Gross and Werner 1978). Today, mullein is found in all 50 of the United States and 10 of 13 Canadian provinces and is listed as a noxious weed in Colorado, Hawaii, and South Dakota (USDA PLANTS database). Mullein populations in western North America are larger, denser, and composed of larger plants than populations found in the European native range (Alba and Hufbauer 2012). Populations are often found in disturbed areas (e.g. following fire), along roadsides and in rocky soils (Gross and Werner1978). Plants are biennial, forming a rosette during the first season and winter of growth and then bolting and flowering in the second season (Gross and Werner 1978). Introduced populations have escaped from several guilds of herbivores found in the native range, and have escaped most of their specialist herbivores (Alba and Hufbauer 2012). Herbivorous insects in the introduced range

include generalist grasshoppers, and two co-introduced specialist species: a thrips and a seed-head weevil (*Haplothrips verbasci* and *Gymnetron tetrum*).

Plant sampling and growth

With the help of colleagues I collected seeds from 12 populations in the native range and 51 populations in the introduced range (Table 3.1). Seeds were collected and mixed from multiple seed capsules per plant but kept separate by maternal plant and stored at 3.4°C until planting. I germinated seeds in germination peat pellets on a mist bench (24.8/19.9°C average day/night temperatures; 59.5/77.4% average day/night humidity). When the seedlings were 6 weeks old they were transplanted into 1-gallon pots with Fafard potting media. All plants were randomized and grown in the Colorado State University greenhouse (21.9/18.4°C average day/night temperatures). Plants were watered as needed, typically every three days, and were fertilized once with Osmocote, a slow-release fertilizer. I grew seven replicates of each maternal line. Three replicates were used for the resistance experiments and four replicates were used for the tolerance experiments.

Insect rearing and collection

All larvae (generalist) were raised from eggs collected from a lab colony of *Trichoplusia ni* (cabbage loopers). Larvae were kept in growth chambers set to a 16/8 light/dark photoperiod and 25.6 degrees Celsius and fed a pinto bean Lepidoptera diet. Third instar larvae were used for each of the experiments. All larvae were starved for 24-26 hours prior to the start of the experiments.

Gymnetron tetrum (commonly referred to as mullein seed-eating weevils), specialist herbivores on mullein that have also been introduced to North America, were collected from a naturalized and unmanaged population of common mullein in Fort Collins, CO. Weevils were starved for 24-26 hours prior to the start of the experiments.

Resistance to herbivory

Specialist herbivore: leaf age

To evaluate whether a specialist weevil differentiates between more highly defended young leaves and less defended older leaves, and whether the degree of differentiation depends upon the origin of the plant (native vs. introduced), I conducted an experiment in which weevils were given a choice between discs of young (fourth whorl of the plant) and old leaves (bottom whorl of the plant) in a Petri dish. Both leaves were from the same plant. Thirty two plants, one from each of 12 native and 20 introduced populations were randomly selected for this experiment. Five *G. tetrum* weevils were placed in a large plastic Petri dish with the two leaf samples. Because there is a substantial size difference between young and old leaves and I did not want the weevils to select a leaf based on size rather than feeding preference, I cut discs from each leaf with a 2-inch diameter biscuit cutter. Leaf discs were placed on moist paper towel in the Petri dish and weevils were allowed to feed for 45 hours. Weevils do not cause easily visible feeding damage on mullein leaves, thus data were collected on where weevils were found (young leaf, old leaf, neither leaf) at 1, 5, 10, 20, 25, 30, and 45 hours. I used a linear mixed model (Proc. mixed) repeated measures analysis with a covariance structure type AR(1) to evaluate if the proportion of time spent on young and old mullein leaves differed, and whether any preference for young vs old leaves differed by the range of the plant's origin. Time, range, leaf age, and the

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interaction between range and leaf age were treated as fixed effects while population of the maternal line nested within range was treated as a random effect in my model. The subject of my repeated measures was the plant. All statistical analyses were carried out in SAS, version 9.3 (SAS, Cary Institute, NC 2010).

Specialist herbivore: plant range

To evaluate whether a specialist weevil showed a feeding preference for plants from the native or the introduced range, I performed a choice test with whole-leaf samples from both ranges. Sixty-eight plants from 12 native and 47 introduced populations were randomly selected for this experiment. Ten *G. tetrum* weevils were placed in a large plastic Petri dish with two similarly sized leaf samples collected from randomly paired plants from the native and introduced range. Leaf stems were wrapped in moist paper towel and the leaves were placed in a dish that was lined with another moist paper towel. Weevils were allowed to feed for 45 hours and were then removed from the dish. Weevil locations (native leaf, introduced leaf, neither leaf) were recorded at 1, 5, 10, 20, 25, 30, and 45 hours and proportional data is presented here. I used a linear mixed model (Proc. mixed) repeated measures analysis with a covariance structure type AR(1) to determine if the weevils preferred native or introduced mullein leaves. Time and range were treated as a fixed effect and population of the maternal line nested within range was treated as a random effect in my model. The subject of my repeated measures was the plant. The PARMS statement was used to specify initial values for the covariance parameters and achieve convergence of the procedure.

Specialist herbivore: whole plant selection

To observe natural levels of colonization and plant choice in a field setting, I moved potted mullein plants outdoors and recorded the number of *G. tetrum* that colonized each plant. Between one and three plants (157 total) from each of 12 native and 47 introduced populations were grown in the greenhouse and then moved outdoors in Fort Collins, Colorado. Plants were randomly organized on the greenhouse bench as well as outdoors and were randomly re-arranged weekly. All plants received ample water as necessary, typically every other day. *G. tetrum* naturally colonized the plants. I recorded weevil preferences twice, four weeks apart. Weevils were not disturbed and the plants were not moved (except for randomizing them) during the time span in which data were collected. I used a linear mixed model (Proc. mixed) repeated measures analysis with a time series structure type AR(1) to determine if the weevils were more abundant on native or introduced mullein plants. Range was treated as a fixed effect and population of the maternal line nested within range was treated as a random effect in my model. The subject of my repeated measures was the plant.

Generalist herbivore: leaf age

Similar to the choice test for leaf age with a specialist insect, I tested for a preference between young leaves and older leaves with a generalist insect. My feeding assay set-up was nearly identical to that with the specialist weevil, as I collected two leaf samples from the same plant, one sample from a young (fourth whorl of the plant) leaf and another from an old (bottom whorl of the plant) leaf. In an effort to standardize the leaf areas and focus the larval choice on palatability and preference, I again cut discs from each leaf with a 2-inch diameter biscuit cutter. I sampled 33 plants from 12 native and 21 introduced populations which were randomly selected

for this experiment. One *T. ni* larva was placed in a large plastic Petri dish with two leaf discs, which were placed on moist paper towel in the dish. Third instar larvae were allowed to feed for 48 hours and were then removed from the dish. Leaf discs were scanned and ImageJ software was used to obtain leaf area remaining measurements (Schneider et al. 2012). I used a linear mixed model (Proc. mixed) to determine if the generalist herbivore preferred young or old mullein leaves and if that preference was different for plants from the native versus the introduced range. Range, leaf age and the interaction between range and leaf age were all treated as fixed effects in the model. Population of the maternal line nested within range was treated as a random effect. Data were log transformed to meet the assumptions of the model.

Generalist herbivore: plant range

To determine the preference of a generalist herbivore and differences in plant defense of plants from the native and introduced range, I carried out a choice test with leaf discs sampled from plants from both ranges. Here again I cut discs from each leaf with a 2-inch diameter biscuit cutter and placed the discs in a dish with one third-instar *T. ni* larva. Leaf discs were collected from the fourth whorl of randomly selected and paired native and introduced plants, one plant from each of 12 native and 35 introduced populations. Larvae were allowed to feed for 48 hours. Leaf disc area remaining was measured using ImageJ software (Schneider et al. 2012). I used a linear mixed model (Proc. mixed) to determine if the generalist herbivore preferred native or introduced plants when presented with the choice. Range was treated as a fixed effect and population of the maternal line nested within range was treated as a random effect. Data were log transformed to meet the assumptions of the model.

Generalist herbivore: caged feeding assay

To evaluate differences in palatability of leaves when the leaves were still connected to the plant, I caged larvae on plants from the native and introduced ranges. Contrary to my choice experiments in Petri dishes, larvae in this experiment were exposed to plant-level constitutive defenses as well as any induced defenses that were triggered within the feeding period. This setup more closely mimics natural herbivore feeding behavior and plant response to herbivory. To set up the experiment I counted 5 whorls down from the newest leaves and randomly selected 2 leaves of approximately the same size and age on each potted plant. I bagged 1 larva on each of the leaves (total of 2 larvae per plant) with mesh cloth bags and zip ties. Larvae fed for 72 hours and lights were on a 14/10 light/dark photoperiod. Leaves were then clipped from the plants and scanned. I used ImageJ to calculate leaf area removed (Schneider et al. 2012). I used a linear mixed model (Proc. mixed) to determine if the generalist herbivore consumed more native or introduced plants. Range was treated as a fixed effect while population of the maternal line nested within range and plant nested within population nested within range were treated as random effects. Data were arcsine transformed to meet the assumptions of the model.

Tolerance to defoliation

Above ground biomass

To measure a second method of plant defense to herbivory, tolerance, I conducted a greenhouse experiment in which I simulated different levels of herbivory and measured the plants' response in biomass. I used 65 sets (12 native, 53 introduced) of 4 sibling plants. Twenty, forty, or sixty percent of each leaf of a rosette (removal amount was constant across leaves of a single plant) was removed with scissors by making a cut perpendicular to the midrib. Control

plants were left untouched. All 260 plants were randomized on the greenhouse benches and continued to be randomized weekly. After 6 weeks, all new-growth above ground biomass was collected from the plants, dried at 80°C for 72 hours, and immediately weighed. I used a linear mixed model (Proc. mixed) to determine plant tolerance to different levels of defoliation. Range (native or introduced), treatment (control, 20%, 40% or 60% above ground biomass removal), and the size (number of leaves) of the rosette prior to imposing the treatment were all treated as fixed effects. Population of the maternal line nested within range was treated as a random effect. Data were log transformed to meet the assumptions of the model.

Following tissue harvest of the different treatments, I removed all remaining above ground biomass from 158 plants that were randomly selected from 60 populations. These plants were allowed to re-grow biomass for a 100% clipped treatment. Plants grew in the greenhouse for 30 days and received water as necessary. All above ground biomass was harvested, dried at 80°C for 72 hours, and immediately weighed. I used a linear mixed model (Proc. mixed) to determine what factors (if any) influenced the plants' ability to re-grow following the assigned treatment and then 100% above ground biomass removal. I treated plant range, original clipping treatment, and the mass of the new growth following the original clipping treatment as fixed effects. Population of the maternal line nested within range was treated as a random effect.

Below ground biomass

Because there may be plant growth differences that are not observed by solely measuring above ground biomass, I measured below ground biomass for my control and 60% clipped treatments. Following collection of above ground biomass in my tolerance experiment, 11 plants from the native range and 23 plants from the introduced range were used to measure below

ground biomass. Above ground biomass had previously been removed at the soil level, leaving the roots fully intact and in soil. Root were soaked in warm water and then washed to remove any remaining debris. Roots were dried at 80°C for 72 hours. Dried roots were collected from the oven and immediately weighed. I measured and compared roots in the control and 60% clipped treatments in an effort to detect any difference in below ground biomass between the two most differing treatments. I used a linear mixed model (Proc. mixed) to determine whether there was a difference in below ground biomass between clipping treatments and if that difference could be attributed to different ranges, the clipping treatment that was imposed, an interaction between treatment and range, the size (number of leaves) of the rosette before the treatment was imposed, or the ability of the plant to grow new tissue post treatment (mass of new growth). These factors were all treated as fixed effects in my model. Population of the maternal line nested within range was treated as a random effect. Data were log transformed to meet the assumptions of the model.

Total biomass

I calculated total biomass for the control plants and the plants in the 60% defoliation treatment, as these were the two treatments for which I had root mass data. I calculated total biomass as the sum of the weight of post-treatment new growth and root weight. I used a linear mixed model (Proc. mixed) to evaluate the effects of plant range, defoliation treatment, and the interaction between range and treatment on the difference in total biomass. These factors were all treated as fixed effects in my model. Population of the maternal line nested within range was treated as a random effect. Data were log transformed to meet the assumptions of the model.

Relationship between resistance and tolerance

I measured a tradeoff between resistance and tolerance using related individuals (e.g. siblings) with resistance measured on different individuals from which tolerance was determined. I evaluated a trade-off between resistance and tolerance with a linear regression. I used above ground biomass measures to plot tolerance as the difference between post treatment tissue growth of damaged and control plants (i.e. tolerance = damaged plant growth – control plant growth). Resistance measures from my bagged experiment with a generalist herbivore were used as the response variable. I used a simple linear regression model (Proc. reg) to determine the relationship between tolerance and resistance of all plants, as well as to determine if the relationship differed between native and introduced plants.

RESULTS

Resistance to herbivory

Specialist herbivore

Specialist weevils preferred to feed on the younger, more defended leaf discs. I found a significant interaction between range and leaf age ($F_{1,413}$ = 0.4.18, *P*=0.0416) such that weevils preferred young leaves from the introduced range, but not for those from the native range (Figure 3.1). The individual effects of time ($F_{1,413}$ = 0.02, *P*=0.8904), range ($F_{1,413}$ = 0.71, *P*=0.3999) and leaf age ($F_{1,413}$ = 2.10, *P*=0.1478) were not significant.

When given a choice between native and introduced leaves, the feeding weevils (omitting those not on leaves) favored introduced leaves, but that trend was not statistically significant ($F_{1,421}$ = 0.60, *P*=0.4407).

In a more natural setting with whole mullein plants kept outdoors, naturally occurring weevils located and colonized the plants. Similar to the leaf choice test, the weevils did not show a preference for native or introduced whole plants ($F_{1,157} = 0.11$, $P=0.7424$).

Generalist herbivore

The generalist herbivore *T. ni* fed significantly more on less defended, old leaves. *T. ni* larvae consumed more of the leaf discs taken from old leaves than the young leaves they were paired with ($F_{1,29} = 20.39$, $P<0.0001$) (Figure 3.2). This trend was consistent across both ranges ($F_{1,31} = 0.87$, $P=0.3589$) and the interaction between range and leaf age was not statistically significant ($F_{1,29} = 0.64$, $P=0.4286$).

I did not find that *T. ni* had a clear preference between native and introduced plants in the larvae feeding trial ($F_{1,45} = 1.96$, $P=0.1688$). Overall, leaf disc damage by the larvae was minimal, with only 2% of native leaf discs and 0.04% of introduced leaf discs consumed.

When larvae were not given a choice of leaf age or plant range and were instead caged on a leaf still connected to a potted plant, *T. ni* fed more on native than introduced plants ($F_{1,3} = 8.89$, $P=0.0585$). Larvae consumed 4.5 times as much leaf tissue of native plants than introduced plants (2.14% versus 9.75%, respectively).

Tolerance to defoliation

Above ground biomass

I did not find a significant difference in the mass of re-growth between any of the treatments ($F_{1,182} = 1.11$, $P=0.2930$) (Figure 3.3) or between native and introduced plants ($F_{1,63} =$

0.14, P=0.7068). I found a general trend by which larger plants prior to manipulation tended to grow more post treatment ($F_{1,182}$ = 3.77, P=0.0538).

Of the 158 plants in my complete defoliation experiment, 25 of them grew back after 100% above ground biomass removal. I did not find a difference between native and introduced plants in their ability to grow back following complete above ground biomass loss ($F_{1,58}$ = 0.29, P=0.5936). The treatment to which each of the plants was previously exposed also did not significantly influence the ability to re-grow tissue ($F_{3,91}$ = 1.16, P=0.3281). Of the plants that grew back, there was no difference in the amount of growth due to range ($F_{1,20}$ = 0.02, P=0.9004), treatment ($F_{1,20}$ = 0.63, P=0.4380), or previous growth ($F_{1,20}$ = 0.30, P=0.5911).

Below ground biomass

I did not find a significant difference in below ground biomass between native and introduced plants ($F_{1,20}$ = 0.29, P=0.5953). The clipping treatment that was imposed ($F_{1,8}$ = 4.05, P=0.0790), an interaction between treatment and range ($F_{1,8}$ = 4.47, P=0.0675), the size (number of leaves) of the rosette before the treatment was imposed ($F_{1,8}$ = 0.26, P=0.6207), or the ability of the plant to grow new tissue post treatment (mass of new growth) ($F_{1,8}$ = 0.65, P=0.4425) did not lead to differences in below ground biomass.

Total biomass

The interaction between defoliation treatment and plant range explains some of the variation in my measures of total biomass ($F_{1,10}$ = 4.43, P=0.0615). Introduced plants had less total biomass (post-treatment growth plus root biomass) than native plants in my control

treatment, but introduced plants had more total biomass than native plants in the 60% defoliated treatment (Figure 3.4).

Relationship between resistance and tolerance

I did not observe a trade-off between resistance and tolerance in either native or introduced plants (Figure 3.5). The coefficient of determination (R^2) was 0.0047 and 0.001 for native and introduced plants, respectively.

DISCUSSION

In evaluating the role of defenses against herbivory in biological invasions it is important to understand defenses broadly, both resistance to specialist and generalist herbivores and tolerance of herbivore damage. Unlike many species in which defenses appear to differ between native and introduced populations, I found native and introduced mullein plants to have comparable defenses with respect to both resistance and tolerance. For the first time in this system I tested both a specialist and a generalist herbivore on the same set of native and introduced plants. We know that this system supports the optimal defense theory (Alba et al. 2013). In my feeding assays with young and old leaf discs and native and introduced plants, the weevils preferred younger leaves from introduced plants. In fact, when I observed naturally colonizing specialist weevils on my outdoor plants, they were consistently found in the upper whorls of the leaves (i.e. the younger, high-iridoid leaves). According to Alba et al. (2012) those leaves are the most highly defended of all. Studies have shown that highly defended young leaves may deter generalist herbivores but may, in fact, attract specialists. It is possible that the

specialist weevils are attracted to the high levels of iridoid glycosides or a different defense compound that is found in younger rosette leaves. Increased defenses in young tissues protect the plants from generalists, the majority of insect herbivores that are present in the introduced range, without incurring the cost of attracting the majority of specialists from which the plants have escaped.

Overall, mullein is resistant to both the specialist and generalist insect herbivores that I tested here. Weevils showed a preference for younger leaves from introduced plants. *T. ni* did not feed differently on leaf discs from the two ranges, but showed a strong preference for older leaves. When bagged on the plant they fed more on native plants. Not finding a difference in *T. ni* preference between native and introduced genotypes when using leaf discs cut from the plants, but finding a difference in amount eaten when using intact plants indicates that some defenses effective against this generalist may be induced.

Mullein is also extremely tolerant to defoliation. Indeed, plants that lost 60% of their above ground biomass at the time of treatment grew a nearly equivalent amount of biomass during the 3 weeks following defoliation as my control plants. Similarities across treatments in above ground post-treatment growth were mirrored below ground as well. I did not see any difference in total root biomass between treatments. Tolerance is a defense that does not differentiate between specialist and generalist herbivores and it could potentially be a rewarding defense for the plant to engage. Tolerance to herbivory/defoliation is an under-studied subject in plant invasion ecology but may be a key factor in invasion success.

There are a few limitations to my experimental design. First, it is possible that the roots of my mullein plants were limited in their growth as they were grown in 1-gallon pots, a smaller area than the plants would have growing in a naturalized population. Perhaps we might see

differences in below ground biomass as a result of my defoliation experiment if the roots were not confined to a pot at all, or if I used larger pots. Additionally, it should be reiterated that I mimicked defoliation by clipping portions of leaves to estimate percent area removed. While this was a controlled and successful method of defoliation of the rosettes, clipping with scissors does not replace true herbivore damage (Agrawal 1998; Karban and Baldwin 1997). I removed a portion of every leaf, but herbivory may be disproportionately distributed on a rosette. Also, instances of herbivore saliva inducing plant defenses are well documented (Karban and Baldwin 1997; Walling 2000) and were not replicated in my experiment. Furthermore, my plants were grown in a high resource, ideal environment in the greenhouse. While this provided my experiment with healthy plants, levels of plant defenses may rely, at least in part, on resource availability (Blumenthal 2006; Ward and Young 2002). The growth conditions of my plants may have limited the degree of differences in resistance and/or tolerance that may otherwise be observable in naturalized conditions.

Studies that have discussed a tradeoff between resistance and tolerance have typically done so using related individuals (e.g. siblings) with resistance measured on different individuals from which tolerance is determined (the same method I have chosen to use here). However, an assumption underlying this approach is that both resistance and tolerance are constitutive traits. Few studies have tested for a trade-off between induced resistance and tolerance (Agrawal et al. 1999; Kempel et al. 2011). It is important to note that while studies of constitutive resistance and tolerance may not have found evidence of a trade-off, that does not mean that one does not exist (Fineblum and Rausher 1995). Additionally, plants in high resource conditions may invest in more than one type of defense. I did not find evidence for a trade-off under my high resource experimental conditions, but given different conditions (Coley et al. 1985), or if I measured at a

different stage of growth (Brandt and Lamb 1994; Weltzin et al. 1998), mullein may show a trade-off in defense.

Evolutionary shifts in plant defense following escape from herbivory are thought to be a crucial component of the success of many introduced plant species. However, 'defense' is a muli-faceted trait, including resistance as well as tolerance to herbivory. Furthermore, defenses against one type of herbivore may be attractants to other herbivores. While resistance is a common first line of defense against attack, tolerance is also an effective method of recovery from herbivory that can alter the survival and success of plant populations. Likewise, while some introduced plants have been released from the top down regulation of specialist herbivores in their new range, not all introduced plant systems may follow that pattern. I used mullein plants from a broad sampling from both native and introduced ranges to determine that both native and introduced plants are highly defended in both resistance and tolerance to herbivory. These high levels of both resistance and tolerance that are consistent across ranges likely contribute to mullein's success in the introduced range.

Table 3.1. *Verbascum thapsus* seed collection locations in the native and introduced ranges. GPS coordinates and site elevation (m) are provided. A subsample of these populations was used in each experiment.

State/country	Site name	Latitude	Longitude	Elevation (m)
Native range				
Czech Republic	Vroutek	50.18052	13.38015	332
Finland	Helsinki	60.206	25.133	19
	Tampere	61.35196639	24.83544139	90
France	La Faurette	45.953333	1.022778	269
Germany	Grißheim	42.888	7.581	204
Romania	Iasi	45.146	27.639	36
Sweden	Tobo, Skolvagen	66.83727	16.02247	1224
	Orbyhus railway station	66.8188	16.03915	872
	Tobo railway station	66.83593	16.01188	1069
	Tegelsmora	66.83685	16.02581	1292
Switzerland	Biere	46.53779	6.332622	699
	Village of Marly	46.847	7.174	583
Introduced range				
Colorado	Lake John	40.781757	-106.478542	2466
	Dadd Gulch	40.699555	-105.544065	2142
	Narrows Campground	40.689931	-105.431767	1968
	Mouth of Poudre Canyon	40.665287	-105.219436	1604

	Steamboat east	40.380543	-106.803849	2240
	Craig	40.514036	-107.621231	1949
	Steamboat west	40.486446	-107.105211	1967
	Hewlett Gulch	40.689393	-105.310408	1748
	Steamboat Middle	40.501103	-106.92329	2011
	Poudre Bike Trail	40.601156	-105.092969	1520
	Golden Gate Canyon	39.8254897	-105.311569	2396
	Hayden	40.491534	-107.315247	1923
	Picnic Rock	40.671292	-105.230554	1606
	Cathy Fromme Prairie	40.510643	-105.099304	1530
Hawaii	Puu Huluhulu hunter station	19.68785	-155.46444	1999
Idaho	Worley	47.554139	-116.916306	781
Illinois	Savoy	40.01649521	-88.2569578	228
Indiana	Etna Green	41.258302	-86.05134	245
Kansas	Manhattan	39.208294	-96.584702	337
Maryland	Edgewater	38.90028	-76.55556	30
Massachusetts	Sutton	42.201024	-71.77729	135
	Newburyport	42.808854	-70.878903	17
	Provincetown, Cape Cod	42.073858	-70.207658	18
Montana	Missoula	46.874115	-115.015724	1644
	RimRock Lane, Great Falls	47.404667	-111.327917	1033
	Rusted Lane	47.488569	-111.224858	1068
	89 South	46.96261111	-110.7555556	1630
	Highwood Rd.	47.432056	-111.319944	1063
	Craig	47.06977778	-111.9719444	1082

|				
	Dry Hollow	46.22212	-111.37338	1379
New Hampshire	Merrimack	42.879325	-71.527547	69
	Portsmouth-1	43.06911	-70.754047	11
	Portsmouth-2	43.059768	-70.803801	23
	Milford	42.84138	-71.67622	78
North Carolina	Raleigh	35.760757	-78.677434	98
Ohio	Bainbridge	41.417436	-81.365826	345
Oregon	Jackson county	42.6638	-122.8263	465
	Wasco county	45.531	-121.0919	239
	Douglas county	42.9259	-123.4901	239
Pennsylvania	Pittsburgh	40.436992	-79.944453	301
Tennessee	Chilhowee	35.557542	-84.010956	273
Virginia	Mountain Lake	37.354023	-80.538698	1219
Washington	Bainbridge Island	47.664243	-122.575908	42
	Bainbridge Island	47.662881	-122.579658	44
Wisconsin	Arkansaw	44.533135	-92.059468	218
	Five-Mile Bluff Prairie	44.533977	-92.05007	214
	Bayside	43.173124	-87.888506	207
Wyoming	Jackson	43.404	-110.751556	1907
	Sweetwater Co.	43.429583	-110.781583	1853
Toronto	UTM	43.55065	-79.65902	106
	Credit Meadows	43.60552	-79.71677	164
	KSR	44.0292	-79.53742	300
	Hewick Meadow	43.56723	-79.68118	137
	Conservation Park	43.6343	-79.73807	172
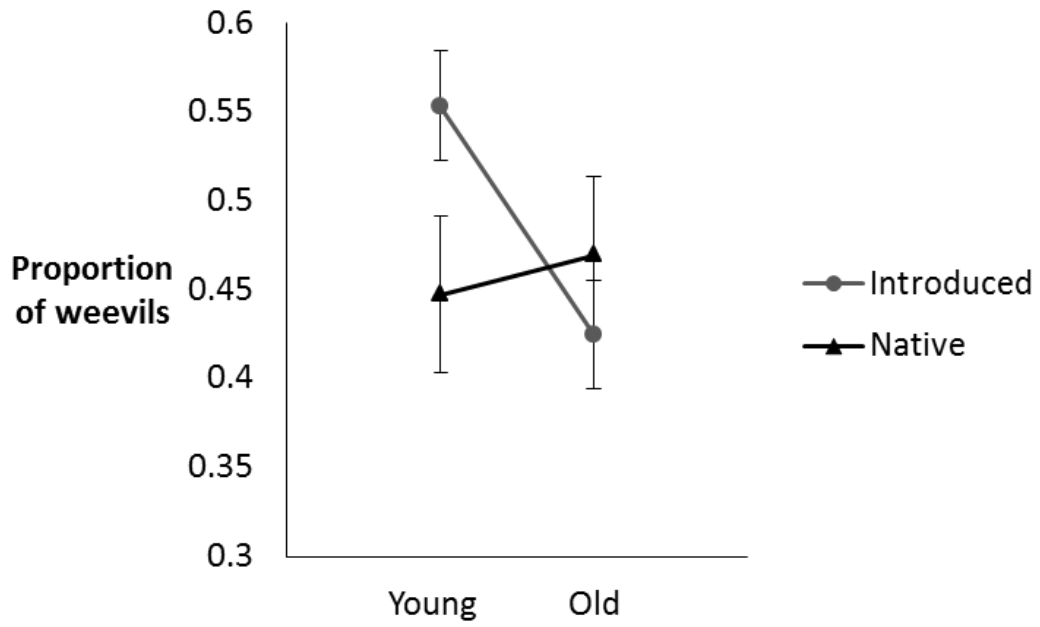
Figure 3.1. Choice test between young and old leaf discs with specialist weevils, *G. tetrum*. There is a significant interaction between range and leaf age ($F_{1,413} = 0.4.18$, *P*=0.0416) such that weevils preferred young leaves from the introduced range. Data are means +/- 1 SE bars.
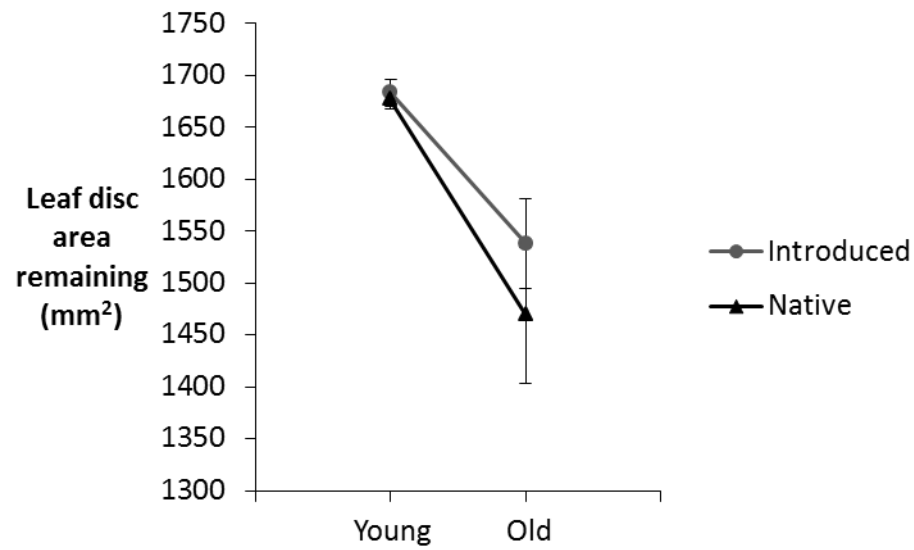
Figure 3.2. Results from the choice test between young and old leaf discs with generalist *T. ni* larvae. Larvae fed more on older leaves in general ($F_{1,29}$ = 20.39, *P*<0.0001), but fed most on older leaves from the native range ($F_{1,45}$ = 1.96, *P*=0.1688). Data are means +/- 1 SE bars.
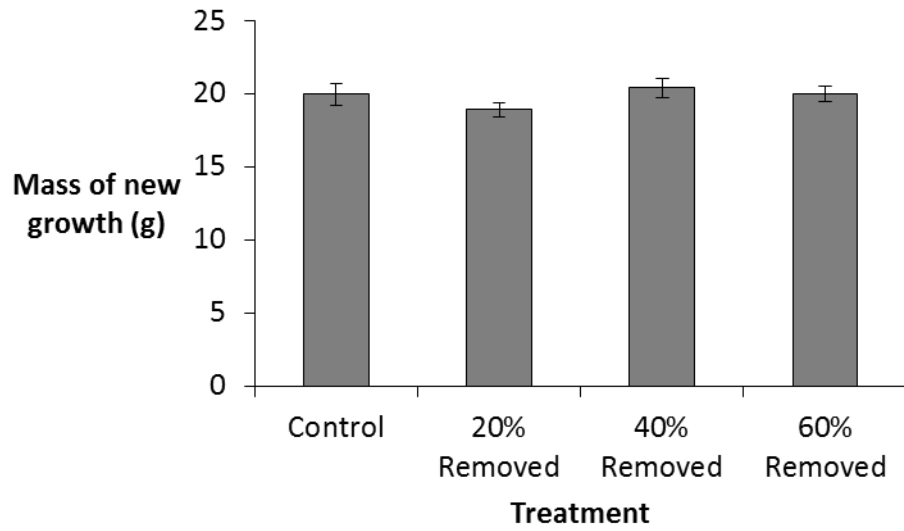
Figure 3.3. Difference between defoliation treatments of the mass of above ground tissue growth post treatment ($F_{1,182} = 1.11$, P=0.2930). Data are means +/- 1 SE bars.

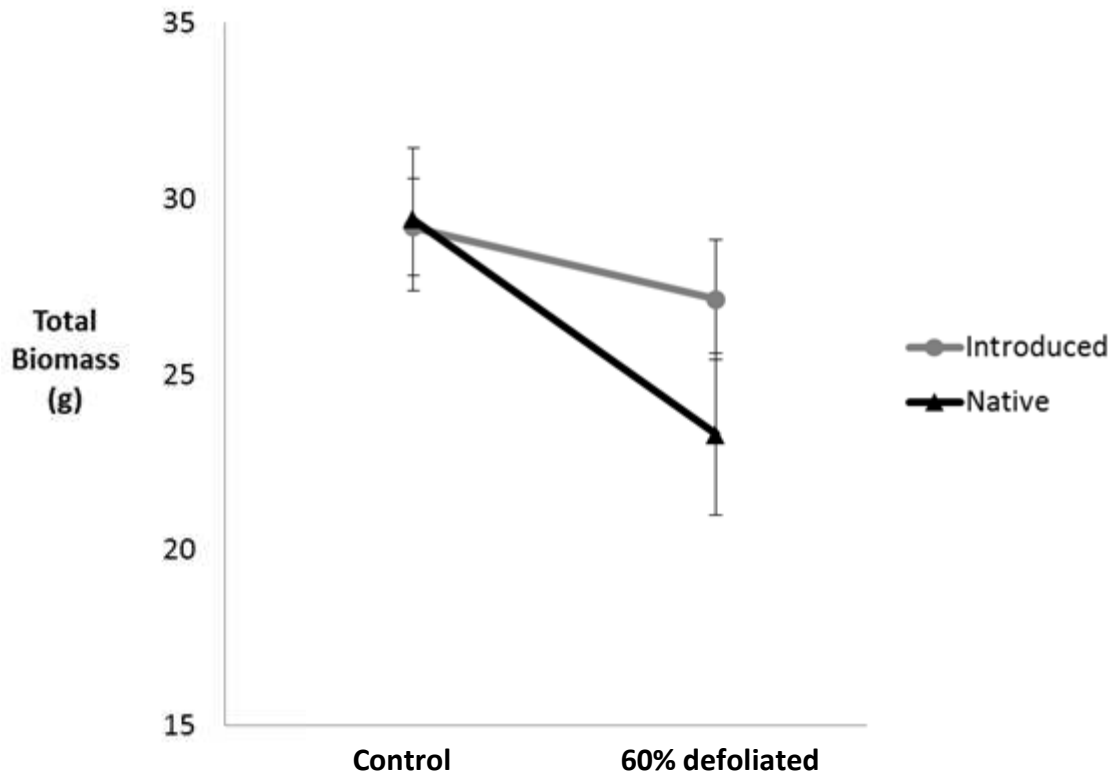Figure 3.4. Total biomass of control plants and the 60% defoliated plants. There is a significant interaction between range and treatment ($F_{1,10} = 4.43$, P=0.0615). Introduced plants had less total biomass (post-treatment growth plus root biomass) than native plants in my control treatment, but introduced plants had more total biomass than native plants in the 60% defoliated treatment. Data are means +/- 1 SE bars.
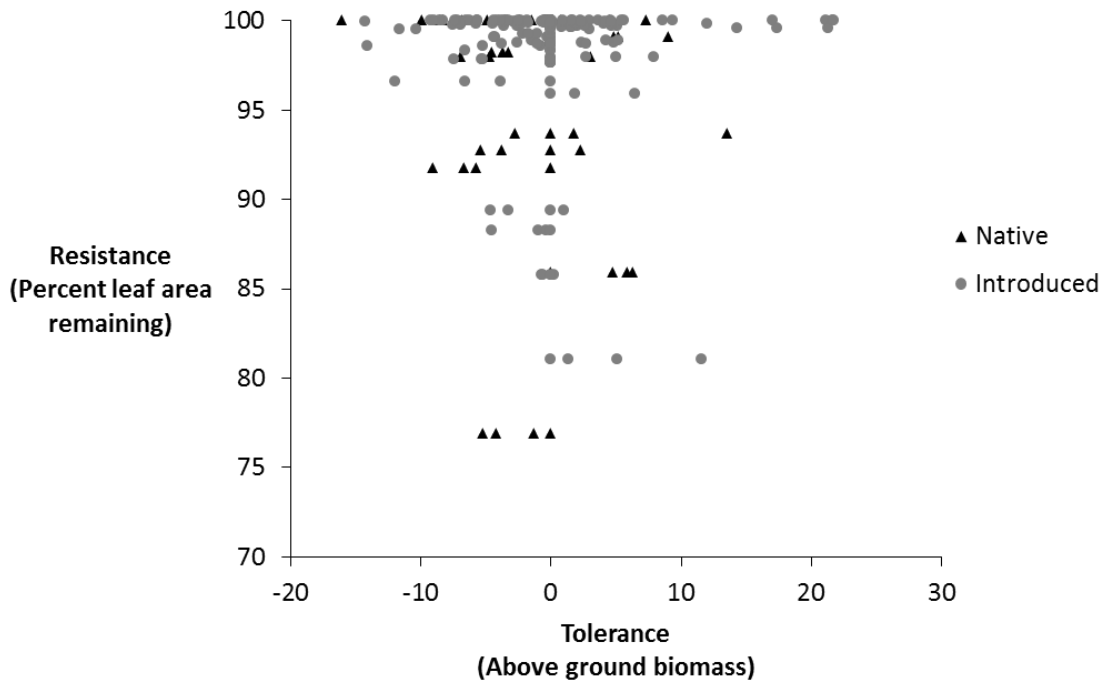
Figure 3.5. No evidence of a resistance and tolerance trade-off. Tolerance is the difference between post treatment tissue growth of damaged and control plants (i.e. tolerance = damaged plant growth – control plant growth). Therefore points plotted at '0' tolerance are control plants. Resistance is reported as the leaf area remaining from the generalist: whole plant experiment, so the level of resistance is positively correlated with the data. The points are replicate sibling plants that were used in both experiments. A trade-off would be depicted by a general trend with negative slope.

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APPENDIX

REPRODUCTIVE STRATEGY, PERFORMANCE, AND POPULATION DYNAMICS OF

THE INTRODUCED WEED BLACK HENBANE (*HYOSCYAMUS NIGER* L.)

Appendix Table 4.1. AIC tables for seed pods

Model for seed pods produced (log transformed)	AICc	Δ_i	Akaike weight (w_i)	Likelihood ratio
1	1736.7	0.0	0.832078276	1
2	1740.7	4.0	0.112609549	0.135335283
3	1742.3	5.6	0.050598732	0.060810063
4	1747.1	10.4	0.004590213	0.005516564
5	1755.1	18.4	8.40727E-05	0.000101039
6	1756.9	20.2	3.41814E-05	4.10796E-05
7	1760.8	24.1	4.86313E-06	5.84456E-06
8	1768.9	32.2	8.47272E-08	1.01826E-07
9	1771.2	34.5	2.68278E-08	3.22419E-08

Appendix Table 4.2. Factors and interactions in each of the 9 models that were tested for seed pod production.

Factors in the model	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7	Model 8	Model 9
Elevation of site				X	X	X	X	X	
Previous year's total growing degree days May 1-Sept 30		X	X		X	X		X	
Previous year's total precipitation May 1- Sept 30			X		X	X		X	
Total growing degree days May 1-Sept 30	X	X	X		X	X	X	X	
Total precipitation May 1-Sept 30	X	X	X			X			
laggdd*lagprecip							X	X	X
gdd*precip									X

Appendix Table 4.3. AIC tables for plant height

Model for plant height (log transformed)	AICc	Δ$_i$	Akaike weight (w_i)	Likelihood ratio
1	978.4	0.0	0.932298177	1
2	984.7	6.3	0.03995096	0.042852127
3	985.5	7.1	0.026779929	0.02872464
4	992.9	14.5	0.000662094	0.000710174
5	995.0	16.6	0.000231692	0.000248517
6	997.2	18.8	7.71235E-05	8.27241E-05
7	1013.4	35.0	2.341E-08	2.511E-08
8	1020.1	41.7	8.21325E-10	8.80968E-10
9	1039.0	60.6	6.46297E-14	6.9323E-14

Appendix Table 4.4. Factors and interactions in each of the 9 models that were tested for plant height.

Factors in the model	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7	Model 8	Model 9
Elevation of site	X	X	X		X		X		
Previous year's total growing degree days May 1-Sept 30	X	X	X			X			
Previous year's total precipitation May 1- Sept 30	X	X	X			X			
Total growing degree days May 1-Sept 30	X	X	X	X	X	X			
Total precipitation May 1-Sept 30		X		X		X			
laggdd*lagprecip			X		X			X	X
gdd*precip									X