INTERACTIONS BETWEEN *MOGULONES CRUCIFER* AND TARGET AND NON-TARGET BORAGINACEAE SPECIES IN MONTANA AND WASHINGTON

by

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ABSTRACT

Invasive plant species can significantly harm the environment and economy. Classical biological control (biocontrol), the deliberate release of a specialized natural enemy from the invasive plant's native range to reduce its abundance or spread in its introduced range, is a tool commonly used to manage invasive plants. However, concerns persist regarding biocontrol agents affecting non-target species. Therefore, my study focused on the root weevil (*Mogulones crucifer* Pallas*)*, approved in Canada as a biocontrol agent for the invasive plant houndstongue (*Cynoglossum officinale L.)*, to assess its effect on its target and closely related non-target plants. Over three years at 14 sites in Montana and Washington, where *M. crucifer* has self-dispersed, I monitored *M. crucifer*'s oviposition and herbivory on *C. officinale* and five non-target species (*Hackelia micrantha* (Eastw.) J.L. Gentry, *Hackelia floribunda* (Lehm.) I.M. Johnson, *Lithospermum ruderale* Dougl. Ex Lehm., *Mertensia ciliata* (James ex Torr.) G. Don, *Mertensia oblongifolia* (Nutt.) G. Don., and an unidentified annual Boraginaceae), tracking the observed plants' growth and demography. Our findings suggest that *M. crucifer* prefers *C. officinale* but can oviposit and feed on the non-target plants. However, these non-target interactions seem unlikely to lead to substantial population declines of these plants. Notably, *C. officinale* populations consistently declined across sites, supporting the effectiveness of *M. crucifer* in suppressing the target species. The limited oviposition and juvenile development on non-target plants align with spillover effects observed in previous studies, where *M. crucifer*'s impact did not extend to non-target population-level changes. However, there were observed declines of some non-target plant populations, yet our models indicated that these declines were not related to *M. crucifer* herbivory or oviposition. Regardless, I recommend further long-term studies to clarify the nature and extent of these population declines where *M. crucifer* interacts with the non-target plant of interest.

CHAPTER ONE

INTRODUCTION

Invasive plant species have a variety of negative effects on the environment, including on native plants, biodiversity, agriculture, forestry, nutrient and fire cycles, water availability, and recreation and tourism (Hejda et al., 2009; Charles & Dukes, 2007; Beck et al., 2008; Pysek et al., 2012). The U.S. spends at least $34 billion a year because of invasive plants (Pimentel et al., 2005). Conventional invasive plant management techniques, including mechanical and chemical control, can be effective in some situations and for some invasive plant species. However, these methods are not always feasible or economically sustainable for management of large infestations in inaccessible terrain or remote areas (Culliney, 2005).

Problematic invasive plant species are successful in their introduced ranges for many reasons, but one that is often mentioned is the enemy release hypothesis (Keane & Crawley, 2002). This hypothesis refers to the species' ability to achieve higher densities when growing in locations without the natural enemies that regulate populations in its native ranges (Keane & Crawley, 2002). The enemy release hypothesis is the basis of classical biological weed control.

Classical biological control of invasive plants (hereafter referred to as biocontrol) is the deliberate release of a specialized natural enemy from the invasive plant's native range to reduce its abundance or spread in its introduced range. The development of biocontrol agents begins with an exploration of the target invasive plant's native range, collecting potential natural enemies to be tested for host-specificity in lab settings and under field conditions.

Host-specificity testing typically begins with no-choice tests, where a potential biocontrol agent is confined to one plant species at a time without being able to exhibit its normal host-selection behavior (Hinz et al., 2014). Additionally, the biocontrol agent will be exposed to choice-tests, where it may be caged in a field setting or under natural field conditions, where it chooses from among the target invasive plant and other possible host plants (Hinz et al., 2014). In more recent years, these tests have been combined with ecological and molecular evaluations (Gaskin et al., 2001). If an agent is found to be host-specific and effective and receives official regulatory approval for field release, the testing is usually followed by importing, rearing, and release from quarantine for establishment in the target invasive plant's introduced range (Suckling & Sforza, 2014).

Host-specificity testing has gone through significant changes since the inception of biocontrol: until 1960 there was either no host-specificity testing or the emphasis was to ensure crop species were not attacked; from 1961 to 1990, there was an increase in testing plants closely related to the target weed to determine the actual host range of the biocontrol agent; and from around 1990 to present, native species in the invaded range have increasingly been included with crop and other beneficial plant species in host-specificity testing (Hinz et al., 2019). The current testing process aims to quantify risks to non-target plant species in the introduced range (Hinz et al., 2014). Host-range testing includes determining the potential biocontrol agent's fundamental and realized host ranges. The fundamental host range includes all plant species in or on which the organism's life cycle can be completed, and the realized host range includes the plant species the organism will use under natural field conditions

(Hinz et al., 2014; Schaffner, 2001). The previously mentioned no-choice tests determine the fundamental host range, and the choice tests aim to determine the realized host range. Post-release studies have demonstrated that the realized host range under field conditions is always narrower than the fundamental host range (e.g., Center et al., 2007; Cristofaro et al., 2013; Dudley & Kazmer, 2005; McFadyen et al., 2002; Medal & Cuda, 2010; Paynter et al., 2004; Pratt et al., 2009).

The Department of Agriculture Animal Plant Health Inspection Service (APHIS) is the regulatory agency in the U.S. responsible for biocontrol agent introductions, and currently, their decisions for approval of field release of biocontrol agents have primarily focused on the fundamental host range of a potential agent (Hinz et al., 2014; Patel-Weynand et al., 2021). This approach potentially overestimates the risks associated with a particular biocontrol agent but is the most conservative (Hinz et al., 2014). Overestimates of risk based solely on the potential biocontrol agent's fundamental host range stem from the ability of the agent to complete its life cycle on a plant under no-choice tests, while the reality of the lack of overlap of the potential agent and host under natural field conditions is disregarded. Differences in phenologies, little or no overlap in actual geographic distributions or habitat requirements, or some combination of both can lead to the low probability of the biocontrol agent encountering a non-target plant in natural settings (Hinz et al., 2014).

Suckling and Sforza (2014) conducted systematic reviews of agents deliberately introduced for biocontrol of invasive plants to determine the magnitude of non-target plant interactions and found significant effects to be rare. Additionally, Hinz et al. (2019) conducted

a review of direct non-target attack from intentionally released biocontrol agents from approximately 1863 to 2008 and found that of the 457 biocontrol agents released, 60 (13.1%) resulted in cases of non-target plant feeding in the field, with 75% of these cases occurring on plant species in the same family as the target weed. Hinz et al. (2019) further specified that non-target attack decreased by 50% for those agents released 1991 to2008 compared to those released before 1960. Based on the decrease in non-target attack documented from 1863 to 2008 and the progress in research and technology associated with the host-specificity testing of potential biocontrol agents, we will likely continue to observe improvements to the environmental safety of biological control (Hinz et al., 2019). Regardless of the low percentage of incidence of non-target impacts, any potential of non-target interaction has led to criticism of biocontrol in recent years (Louda & Stiling, 2004; Havens et al., 2019). This is reasonable criticism, considering that once an agent is established, we will not likely be able to remove it from the system.

Havens et al. (2019) raised concerns of the previously mentioned review of non-target plant impact (Suckling and Sforza, 2014). Specifically, Havens et al. (2019) stated that the review was not based on experiments designed to test for non-target impacts but rather on host-range expansion. Havens et al. (2019) pointed out that non-target interactions are likely to go unnoticed and/or unreported unless they are specifically being looked for, occurring on a plant of ecological or economic benefit, or are resulting in significant devastation to a species. Researchers have stated the need for increased post-release monitoring to assess non-target plant use (Blossey et al., 1994; Sheppard et al., 2005). Signs of impact to non-target plants

from biocontrol agents can be subtle, and because it may take years for effects to be detected, monitoring protocols need to incorporate the site of the release as well as regional searches over multiple years to increase confidence of lack of sustained or increased non-target attack (Havens et al., 2019). Currently, most financial investment for biocontrol is spent on developing biocontrol agents. Increases in post-release monitoring will require additional funding, but it should be considered as important as pre-release, host-specificity testing (Hinz et al., 2019).

Catton et al. (2015) conducted one of the few studies on post-release effects on a non-target species. Specifically, they performed a manipulative field experiment with the root weevil *Mogulones crucifer* in southern Alberta, Canada. Canada approved *M. crucifer* as a biocontrol agent for the invasive species *Cynoglossum officinale* in 1997. *Cynoglossum officinale* takes advantage of disturbance to invade a variety of habitats but does exceptionally well in forest clearings and readily spreads by both domestic and wild animals (Winston et al., 2016). *Mogulones crucifer* has readily self-dispersed, effectively and rapidly suppressing *C. officinale* infestations (De Clerck-Floate et al., 2005; DeClerck-Floate & Wikeem, 2009). After *Cynoglossum* spp*., M. crucifer* shows the highest preference for *Hackelia* spp. Therefore, Catton et al. (2015) focused their non-target impact study on *Hackelia micrantha*.

Catton et al. (2015) released weevils on range sites with naturally-occurring populations of *H. micrantha* growing interspersed with *C. officinale* or in near absence of *C. officinale*. Herbivory was monitored for three years after release, and at the end of the third-year plants were destructively sampled and dissected in search of *M. crucifer* eggs and larvae.

After two years, weevils were not detected on sites without *C. officinale,* and non-target feeding was restricted to rare, low-level spillover. Catton et al. (2015) defined spillover as non-target adult feeding, or larval development that occurred due to high agent density supplied by local target plants or recent releases. They concluded that non-target, population-level impacts from weevil spillover is unlikely. Additionally, Catton et al. (2015) concluded that, given *M. crucifer*'s relative low host-specificity and *H. micrantha* being one of its most preferred non-target hosts, their findings were likely relevant to other non-target plants and even other biocontrol systems.

The effective suppression of *C. officinale* seen in western Canada since the release of *M. crucifer* in 1997 has led to increased interest from land managers in Montana to use biocontrol for management of this Montana state-listed noxious weed. However, *M. crucifer* was not approved for release in the U.S. due to perceived risks to non-target plants within the same family as *C. officinale*, Boraginaceae. In host-specificity testing, *M. crucifer* was found to be able to develop on 9 of the 22 North American species tested (De Clerck-Floate & Schwarzlander, 2010). However, the rates of development were generally much lower on the non-targets than on *C. officinale,* and *C. officinale* was always the preferred host when *M. crucifer* was given a choice between a non-target and *C. officinale* during host-specificity testing (De Clerck-Floate & Schwarzlander, 2010). Due to concerns of non-target impact and in response to reports that the weevil had become established in the U.S. in Washington, Idaho, and Montana, a federal pest alert was issued in 2010 (USDA-APHIS, 2010), making it illegal to facilitate the distribution of this insect.

In general, evaluating a biocontrol agent's interactions with non-target plants post-release will provide additional support for the safety and efficacy of classical biological weed control. Further, evaluating interactions between unapproved biocontrol agents and non-target plants in areas where the insect has been found to occur is increasingly important, especially when there are existing concerns of effects to plants other than the target weed. *Mogulones crucifer* and the non-target plants of concern in the U.S. are a prime example of a system that should be evaluated for detrimental interactions.

Currently, *M. crucifer* is known to have naturally moved into Washington, Idaho, Montana, Wyoming, and Oregon from western Canada. In 2020, when this monitoring project began, it was known to have established in Washington, Idaho, and Montana. Given that *M. crucifer* oviposits and feeds on other members of the Boraginaceae family in Alberta and British Colombia, Canada (Andreas et al., 2008; Catton et al., 2015), I was interested in determining if similar interactions with non-target plants were occurring in the U.S. Further, Catton et al. (2016) conducted a detailed demographic study focused on the interactions of *M. crucifer* with the non-target *H. micrantha* and found that the herbivory inflicted and larval development that occurred by *M. crucifer* on *H. micrantha* did not lead to population-level impacts. However, Catton et al. (2016) cautioned that the results of their research were not necessarily applicable to other regions and/or non-target species. Therefore, I was interested in determining if interactions (oviposition and herbivory) between *M. crucifer* and *C. officinale,* as well as *M. crucifer* and non-target plants, are correlated with changes in the observed plant's population. Specifically, I examined if the reduction of *C. officinale* plant counts was more

pronounced than the reduction of non-target plant counts and whether this reduction was associated with increased levels of oviposition and herbivory. I also intended to determine if there were differences in the frequency of *M. crucifer* juveniles observed in dissected target versus non-target plants. I hypothesized that *C. officinale* populations would decline more than non-target plant populations and that the reduction would be correlated with increased levels of oviposition and herbivory. Additionally, I hypothesized that more *M. crucifer* juveniles would be observed in dissected *C. officinale* plants than in dissected non-target plants.

CHAPTER TWO

MATERIALS AND METHODS

Study Species

Cynoglossum officinale. *Cynoglossum officinale* L. (Boraginaceae) is an herbaceous, biennial or short-lived perennial native to Eurasia. Plants produce rosettes from a stout taproot in the first year and typically flower in the second or third year (Wesselingh et al., 1997). *Cynoglossum officinale* was first recorded in North America in Ontario in 1859 (Winston et al., 2016) and is a significant problem in disturbed sites throughout western North American grasslands, rangelands, and forests (Dickerson & Fay, 1982). The nutlets containing seeds produced by *C. officinale* are covered with tiny hooks, enabling them to easily attach to animals and people and spread rapidly. Due to its rapid spread, ability to invade areas with a wide variety of conditions, and the plant's toxicity to livestock and humans, *C. officinale* is considered an economically and ecologically significant rangeland weed in the western U.S. and Canada.

Mogulones crucifer. *Mogulones crucifer* Pallas (Coleoptera: Curculionidae) is a root-feeding weevil native to Eurasia, where its primary host is *C. officinale*, with the most damage inflicted through larval feeding within the roots (Prins et al., 1992). Overwintering adult weevils emerge from the soil litter in the spring and feed on *C. officinale* rosettes before mating and ovipositing on *C. officinale* stems, leaf petioles, and roots (Winston et al., 2016). Females prefer to lay eggs on bolting plants over rosettes and large plants over small ones (Schwarzlaender, 1997). However, they will lay eggs on rosettes and small plants if bolting and large plants are not available. Oviposition on the stems and leaf petioles leave distinct black bumps. Larvae move into the roots to feed and develop through three instars (Winston et al., 2016). Mature larvae exit roots to pupate in the soil. In addition to the generation of adults that emerge in the spring/early summer, adults may also emerge in late summer/early fall, feed on *C. officinale* foliage, mate, and lay eggs before winter (Winston et al., 2016). On average, it takes 88-103 days from egg laying to adult emergence (Schwarzlaender, 1997). Overwintering can occur as mature adults in the soil, pupae and new adults in cocoons, or larvae in roots. Adults may live for up to two years, leading to the possibility of overlap in generations and the ability to find larvae throughout the year (Winston et al., 2016). However, most adults perish within one year (Schwarzlaender, 1997). *Mogulones crucifer* can reach high population numbers within several generations when not limited by *C. officinale* availability (Schwarzlaender, 1997).

Study Sites

Due to the inability to move *M. crucifer* (USDA-APHIS, 2010), sites were selected where 1) *M. crucifer* had naturally established, 2) the target plant, *C. officinale,* was relatively

abundant, and 3) a non-target, confamilial plant species was also relatively abundant within the same area. The non-target, confamilial plant species that were identified growing intermixed with *C. officinale* and in the presence of *M. crucifer* included *Hackelia micrantha* (Eastw.) J.L. Gentry*, Hackelia floribunda* (Lehm.) I.M. Johnson*, Lithospermum ruderale* Dougl. ex Lehm.*, Mertensia ciliata* (James ex Torr.) G. Don, *Mertensia oblongifolia* (Nutt.) G. Don., and an unidentified annual Boraginaceae. *Hackelia micrantha* is a native perennial found in western Canada and the western U.S., often in a variety of habitats but generally preferring mesic sites. *Hackelia floribunda* is a perennial that is native to much of the western half of Canada and the western U.S., often found in areas that are wet in the spring (e.g., meadows, wetlands, riparian areas). *Lithospermum ruderale* is a perennial that is native to western Canada and the western U.S. and can be found growing in a variety of habitats. *Lithospermum ruderale* was only present at one study site, the Perry site, with only three total plants observed. Due to the low number of observations, *L. ruderale* was not analyzed. However, the dissection (one plant) data are included in Table 4. *Mertensia ciliata* is a perennial, native to the western U.S. and can be found in moist habitats. *Mertensia oblongifolia* is a perennial, native in parts of the western U.S. and can be found in a variety of habitats from valleys to alpine habitats. An annual Boraginaceae was the non-target of interest at one site, the East Fork Annual site, and while I intended to identify this plant in the third year of the study, it was no longer present in the plot. A nearby Boraginaceae was keyed to *Lappula squarrosa* (Retz.). However, I cannot be sure that was what was growing in the plot,

and therefore, I will refer to the non-target at the East Fork Annual site as annual Boraginaceae.

Fourteen sites (Table 1) in Montana and Washington were revisited for three consecutive years, repeating observational data collection from 2020 to 2023 (initial monitoring began at some sites in 2020 and others in 2021) to collect plant data and document signs of *M. crucifer* herbivory and oviposition.

At each site, a 5m x 5m plot was marked with corner posts, and tape measures were used to divide the 5m x 5m plot into 25, 1m x 1m subplots for ease of data collection and plant mapping. Data on plant height, growth stage (rosette, bolting, or bolting but not flowering), number of flowers, herbivory damage (suspected to be from *M. crucifer*), and *M. crucifer* oviposition scarring were collected for each *C. officinale* and the non-target plant(s) found within the plot for three consecutive years. Herbivory injury was recorded as one of four categories: absent – no sign of herbivory, low – trace amount of herbivory, medium – more than trace but less than 25% of foliage had at least one herbivory mark, and high – more than 25% of foliage had at least one herbivory mark. Oviposition scarring was also recorded as one of four categories: absent – no oviposition scarring, low – 1-9 oviposition scars, medium – 10-20 oviposition scars, and high – more than 20 oviposition scars. Data collection occurred from late June to mid-July, which is near the end or the end of adult weevil activity (oviposition and herbivory), with the intent of capturing the full extent of oviposition and herbivory on targets and non-targets at each site. The number of individuals varied greatly from site to site, with 8-138 *C. officinale* individuals, 13-102 *H. micrantha* individuals,

Table 1. Study sites with associated physical characteristics and non-target plant species.

Site	Longitude	Latitude	Elevation (m)	Non-target Plant(s)
Montana Sites				
Hyalite	-111.007778 W	45.536944 N	1932	*Hackelia micrantha* & *Mertensia ciliata*
Potosi	-111.9170738 W	45.5726403 N	1929	*Hackelia micrantha* & *Mertensia oblongifolia*
Bear Trap	-111.649329 W	45.454443 N	1522	*Hackelia floribunda*
Upper Sureshot	-111.9058236 W	45.5498177 N	2245	*Hackelia micrantha*
Lower Sureshot	-111.8824197 W	45.5405596 N	2141	*Hackelia micrantha*
Perry	-112.99086 W	46.766621 N	1902	*Hackelia micrantha* & *Lithospermum ruderale*
East Fork Hackelia	-113.3884713 W	46.1356865 N	1822	*Hackelia floribunda*
East Fork Annual	113.3887687 W	46.1350999 N	1822	Annual Boraginaceae
Howard Hackelia	-114.543625 W	46.774754 N	1370	*Hackelia micrantha*
Howard Mertensia	-114.516355 W	46.7793 N	1328	*Mertensia ciliata*
Garnet	-113.338417 W	46.82466 N	1793	*Hackelia micrantha*
Washington Sites				
Haberman	-120.625677 W	47.308496 N	1316	*Hackelia micrantha*
Hellebore	-120.609943 W	47.275164 N	1311	*Hackelia micrantha*
Bower's Road	-120.567601 W	47.184454 N	1441	*Hackelia micrantha*

7-105 *M. ciliata* individuals, 55-937 *H. floribunda* individuals, 141 annual Boraginaceae individuals (only found in one site), and 3 *L. ruderale* individuals (only found at one site) observed within the plots the first year of monitoring. Last, I mapped the location of each surveyed plant within the plot to track the growth stage of individual plants over the three years.

Plant Dissections

After the third and final year of observational data collection, I destructively sampled four of each non-target plant(s) and one *C. officinale* from each site. Due to limited numbers of individuals in the third year, one L. ruderale and three annual Boraginaceae plants were taken for dissection. Plants were dissected to inspect for *M. crucifer* eggs and larvae. The number of eggs and larvae found within an individual plant were summed and recorded as total juveniles. The developmental stage and location of all identified eggs and larvae were recorded. Non-target plants selected for dissection were chosen based on observation of high levels of herbivory and/or oviposition and proximity to *C. officinale* within the plot in the third year of observation. Bolting or, if none were bolting, the largest *C. officinale* individuals were selected for dissection.

Data Analysis

All data analyses were performed using R (version 4.3.2). ChatGPT 4o mini was used to aid in writing code to conduct analyses in R, resolve errors from R output, and interpret R analyses output. Non-target species observed at multiple sites were grouped by species for analyses. *Cynoglossum officinale* analyses were conducted using the same site groupings as the non-target species; *C. officinale* pooled across all 14 sites was also analyzed.

In the field, oviposition and herbivory were recorded as a category (absent, low, medium, high). To quantify these categories, I assigned each one a midpoint value; for example, “low oviposition” (1-9 scars) was assigned a value of five. Subplot-level averages were then calculated, and these averages for oviposition and herbivory were used in all analyses.

An analysis of variance (ANOVA) was used to examine the effect of sample year on total plant counts, average oviposition, and average percentage herbivory by species. When significant models were found, a Tukey Multiple Comparison Test was performed at a 95% family-wise confidence level to compare the difference in means among the three response variables (i.e., plant count, mean oviposition, and mean percentage herbivory at the subplot level).

To examine whether plant counts for both non-target species and *C. officinale* was influenced by sample year, oviposition, and herbivory, I used a generalized linear mixed model (GLMM) using a negative binomial distribution with a log link function. Fixed effects included sample year, average oviposition, and average herbivory. When species observations were analyzed from multiple sites, a random effect for site was included to capture unobserved variability across sites.

Last, total number of *M. crucifer* juveniles found during dissections by plant species was modeled with an ANOVA. A Tukey Multiple Comparison Test was performed at a 95% family-wise confidence level to compare the mean number of *M. crucifer* juveniles found during non-target and *C. officinale* plant dissections across species. Non-target species *L. ruderale* and the annual Boraginaceae were excluded from the analysis, as no *M. crucifer* juveniles were found using these species.

CHAPTER THREE

RESULTS

Difference in mean plant counts, oviposition, and percent herbivory per subplot

The ANOVA model did not indicate any significant relationships between non-target plant counts per subplot and sample year ($P > 0.05$), but plant counts per subplot for most of the *C. officinale* groupings were affected by sample year, including *C. officinale* pooled across sites ($P < 0.001$), *C. officinale* at *H. micrantha* sites ($P < 0.001$), and *C. officinale* at *M. oblongifolia* sites ($P < 0.001$). When *C. officinale* was pooled across sites the mean plant count was about 3.5 plants per subplot in year one, in year two plant counts decreased to about 2.0 plants per subplot, and by year three the mean plant count was about 1 plant per subplot (Table 2, Figure 1). *Cynoglossum officinale* plant counts at the *H. micrantha* sites followed a similar trend with about 3.9 plants per subplot in year one, about 2 plants in year two, and about 1.1 plants in year three (Table 2, Figure 2). There was a more dramatic decrease in *C. officinale* mean plant counts per subplot at the *M. oblongifolia* site than the other differences in *C. officinale* plant counts observed with about 5.0 plants per subplot in year one, about 1.4 plants in year two, and about 0.1 plants found in year 3 (Table 2, Figure 3).

Although oviposition was observed on all non-target species, the mean level of oviposition on *C. officinale* was generally higher than the level on non-target at the same sites (Table 2). The ANOVA model evaluating the difference in mean level of oviposition indicated that non-target species *H. micrantha* ($P < 0.001$), *M. ciliata* ($P < 0.001$), and annual Boraginaceae ($P < 0.001$) experienced significant differences in mean oviposition over sample years. *Hackelia*

micrantha oviposition was about 0.6 to 0.7 scars per plant per subplot in year one and year two and increased to about 1.5 in year three (Figure 4, Table 2). *Mertensia ciliata* demonstrated a similar trend with higher oviposition in year three than either year one or two (Figure 5). Oviposition on annual Boraginaceae peaked in year two at about 2.2 scars per plant per subplot (Figure 6). *Cynoglossum officinale* at *M. ciliata* sites ($P = 0.032$) and the annual Boraginaceae site ($P = 0.002$) also experienced a significant difference in mean oviposition over sample years (Figures 4-6, Table 2). Mean oviposition on *C. officinale* at *M. ciliata* sites steadily increased over the sample years with about 1.2 scar per plant per subplot in year one, nearly 3.0 in year two, and about 4.8 scars in year three. *Cynoglossum officinale* at the annual Boraginaceae site experienced the highest level of oviposition at about 8.6 scars per plant per subplot in year one, decreased to 0.0 in year two, and increased in year three to about 1.9 scars per plant. There was no change in mean oviposition for *C. officinale* pooled across sites ($P = 0.898$), with a relatively consistent mean level of oviposition of approximately two oviposition scars per plant per subplot for all three years (Table 2).

Like oviposition, herbivory was observed on all non-target species, with the mean percentage of herbivory generally being higher on *C. officinale* than the paired non-target in years one and two; in year three, however, the percentage of herbivory tended to be higher on the non-target species (Table 2). The ANOVA model evaluating the difference in mean percentage of herbivory per subplot indicated that non-target species *H. micrantha* ($P < 0.001$), *M. ciliata* ($P < 0.001$), and annual Boraginaceae ($P < 0.001$) experienced significant differences in mean percentage of herbivory over the sample years. *Cynoglossum officinale* pooled ($P < 0.001$), at *H.*

Table 2. Mean plant count per subplot, oviposition per subplot, and percent herbivory per subplot ± 1 standard error by species for sample years 1-3. Shaded and non-shaded bands inform the non-target (above) sites that the *Cynoglossum officinale* data are associated with, pooled *C. officinale* does not have an associated non-target pair.

	Mean (±SE) Plant Count/Subplot			**Mean (±SE) Oviposition/Subplot**			**Mean % (±SE) Herbivory/Subplot**		
Species	**Year 1**	**Year 2**	**Year 3**	**Year 1**	**Year 2**	**Year 3**	**Year 1**	**Year 2**	**Year 3**
Cynoglossum officinale (pooled)	3.49 ± 0.45	1.97 ± 0.21	1.01 ± 0.15	2.04 ± 0.28	2.03 ± 0.30	1.87 ± 0.27	31.84 ± 1.90	29.42 ± 2.25	18.33 ± 2.18
Hackelia micrantha	2.77 ± 0.25	3.13 ± 0.30	2.40 ± 0.25	0.71 ± 0.12	0.60 ± 0.11	1.47 ± 0.19	20.72 ± 1.71	32.59 ± 2.29	34.07 ± 2.62
C. officinale	3.93 ± 0.60	1.97 ± 0.24	1.08 ± 0.18	1.35 ± 0.16	2.18 ± 0.35	1.9 ± 0.31	29.25 ± 2.05	32.45 ± 2.65	18.27 ± 2.40
Hackelia floribunda	26.61 ± 11.00	11.44 ± 4.28	1.27 ± 0.48	2.03 ± 0.23	1.82 ± 0.19	1.36 ± 0.20	17.86 ± 1.94	13.42 ± 3.11	10.23 ± 5.62
C. officinale	2.53 ± 0.49	1.93 ± 0.49	1.27 ± 0.38	3.59 ± 1.44	0.83 ± 0.49	2.12 ± 0.75	47.39 ± 5.25	30.58 ± 6.59	13.07 ± 7.27
Mertensia ciliata	3.76 ± 0.86	4.11 ± 0.76	3.22 ± 0.66	0.45 ± 0.23	0.07 ± 0.04	1.57 ± 0.31	12.61 ± 2.06	4.94 ± 0.93	41.15 ± 4.21
C. officinale	7.73 ± 3.79	4.91 ± 1.05	1.09 ± 0.41	1.2 ± 0.34	2.96 ± 0.90	4.75 ± 1.40	21.16 ± 4.36	33.64 ± 5.49	28.98 ± 7.78
Mertensia oblongifolia	1.17 ± 0.17	1.3 ± 0.20	1.50 ± 0.27	0.00 ± 0.00	0.71 ± 0.71	0.00 ± 0.00	2.08 ± 2.08	16.07 ± 10.37	15.63 ± 9.67
C. officinale	5.00 ± 0.67	1.43 ± 0.37	0.07 ± 0.07	1.81 ± 0.30	3.77 ± 1.42	1.07 ± 1.07	13.01 ± 2.52	34.91 ± 8.94	5.36 ± 5.36
Annual Boraginaceae	15.67 ± 6.45	5.00 ± 3.00	2.31 ± 1.33	0.11 ± 0.11	2.19 ± 0.31	0.00 ± 0.00	12.62 ± 4.13	5.47 ± 0.78	57.50 ± 10.90
C. officinale	1.50 ± 0.58	0.08 ± 0.08	0.92 ± 0.38	8.60 ± 2.75	0.00 ± 0.00	1.86 ± 0.82	42.19 ± 10.86	1.04 ± 1.04	37.50 ± 11.31

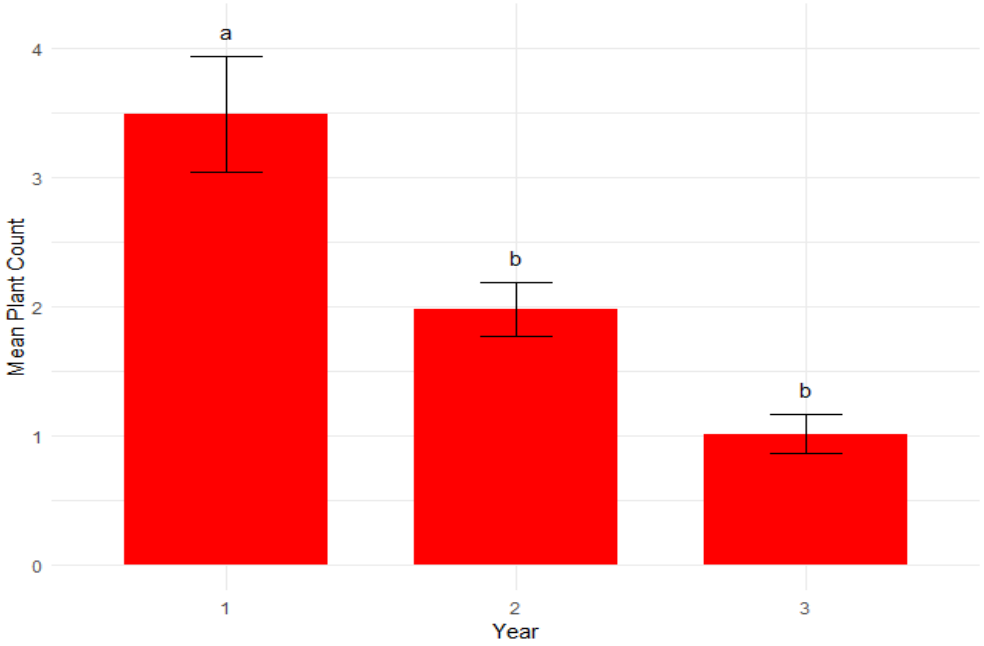

Figure 1. Mean plant counts per subplot over the three sample years for pooled *Cynoglossum officinale* (all 14 sites). Error bars represent 1 standard error. Letters denote differences in means within a single species across years ($\alpha = 0.05$).

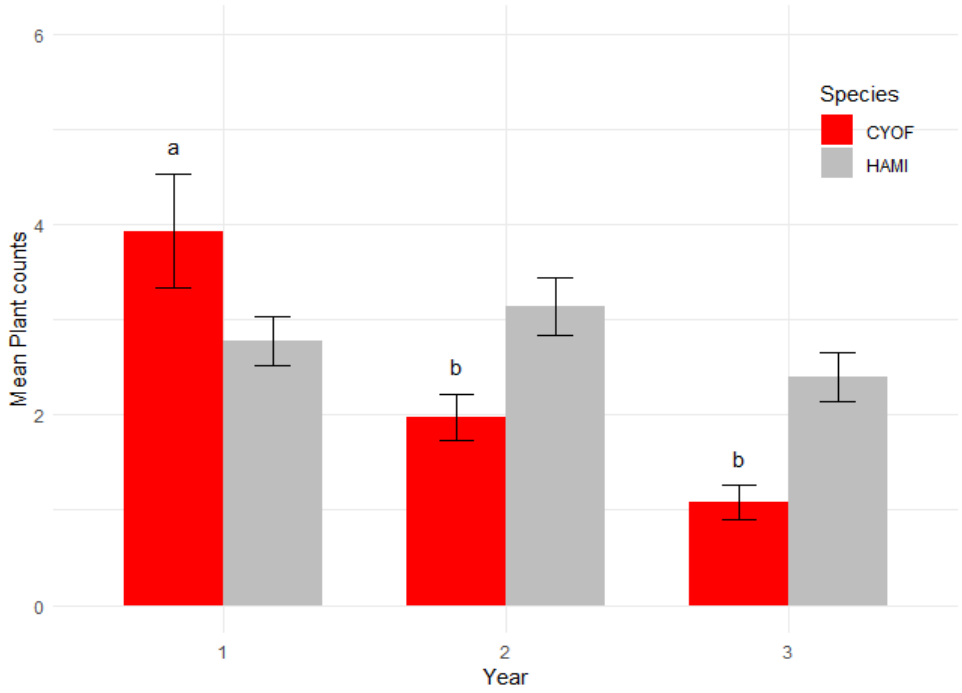

Figure 2. Mean plant counts per subplot for *Cynoglossum officinale* (CYOF) and *Hackelia micrantha* (HAMI) over the three sample years at the *H. micrantha* sites (10). Error bars represent 1 standard error. Letters denote differences in means within a single species across years ($\alpha = 0.05$).

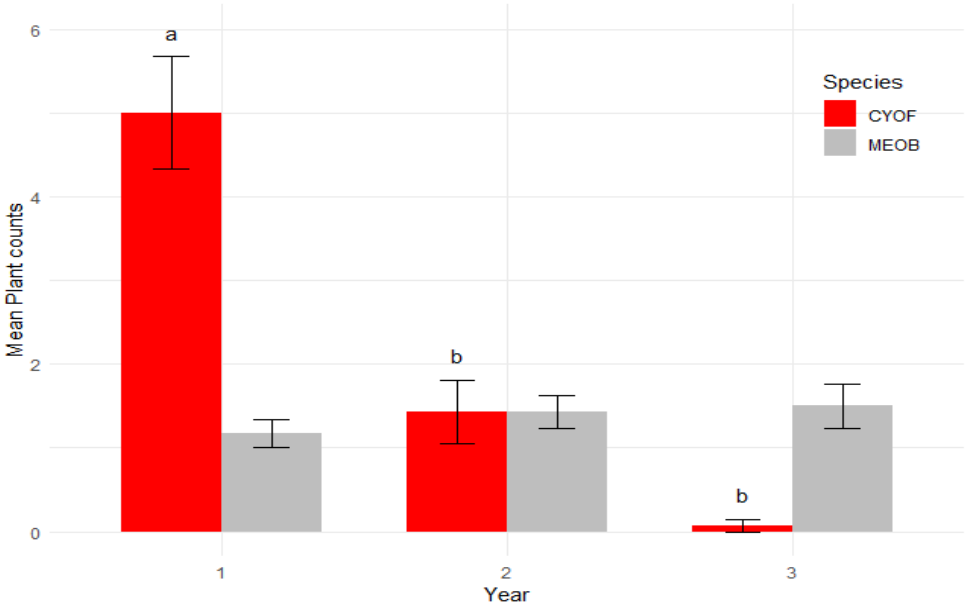

Figure 3. Mean plant counts per subplot for *Cynoglossum officinale* (CYOF) and *Mertensia oblongifolia* (MEOB) over the three sample years at the *M. oblongifolia* site. Error bars represent 1 standard error. Letters denote differences in means within a single species across years (α = 0.05).

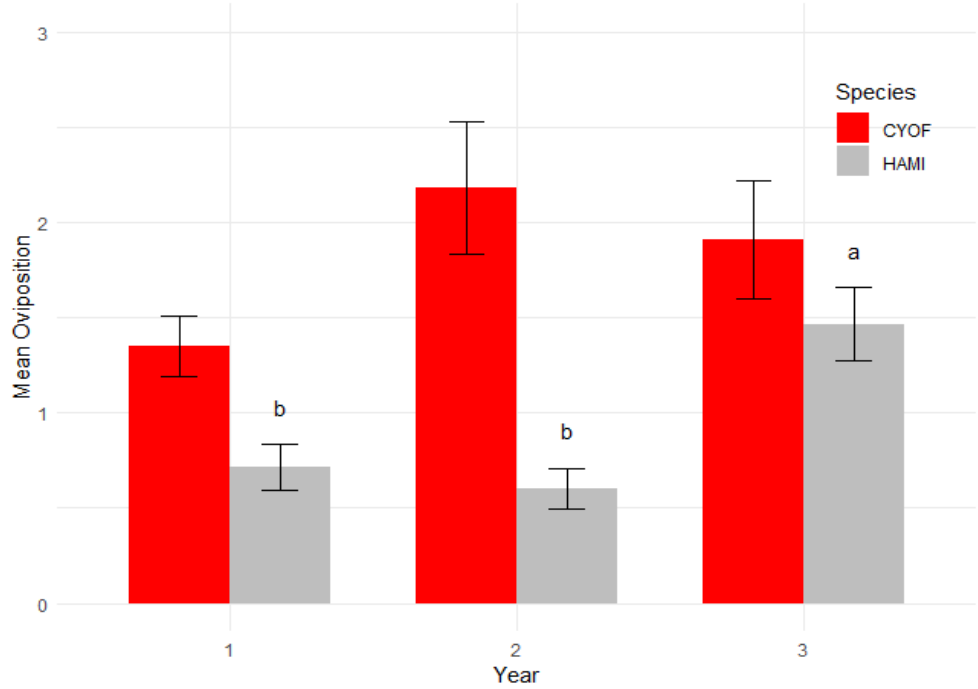

Figure 4. Mean oviposition per plant per subplot for *Cynoglossum officinale* (CYOF) and *Hackelia micrantha* (HAMI) over the three sample years at *H. micrantha* sites (10). Error bars represent 1 standard error. Letters denote differences in means within a single species across years (α = 0.05).

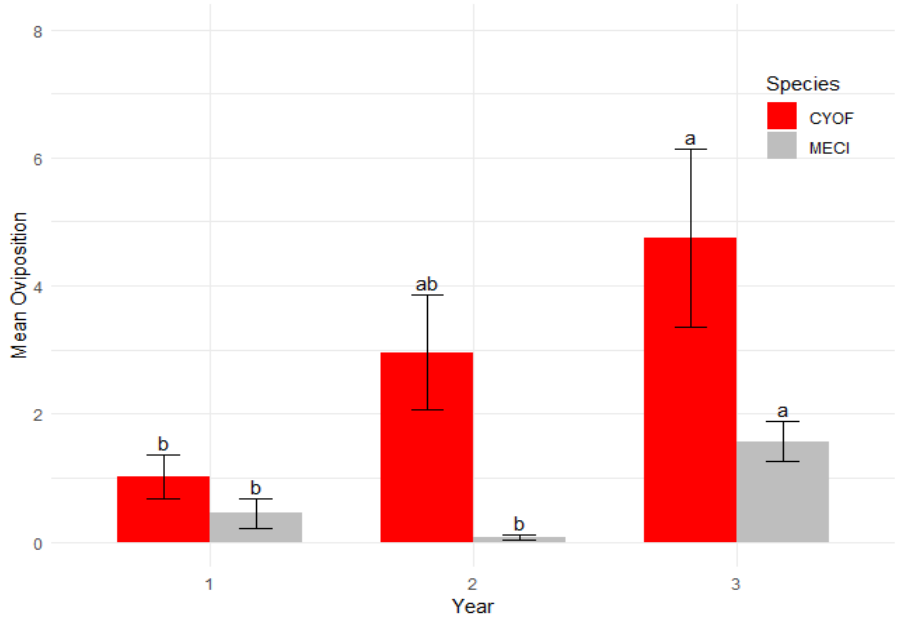

Figure 5. Mean oviposition per plant per subplot for *Cynoglossum officinale* (CYOF) and *Mertensia ciliata* (MECI) over the three sample years at *M. ciliata* sites (2). Error bars represent 1 standard error. Letters denote differences in means within a single species across years (α = 0.05).

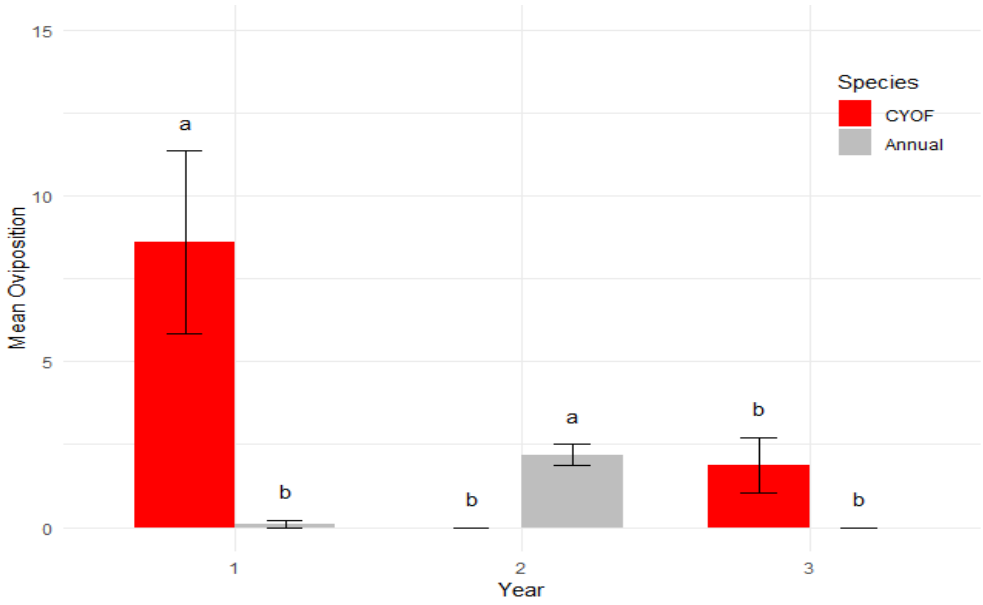

Figure 6. Mean oviposition per plant per subplot for *Cynoglossum officinale* (CYOF) and annual Boraginaceae (annual) over the three sample years at the annual Boraginaceae site. Error bars represent 1 standard error. Letters denote differences in means within a single species across years (α = 0.05).

micrantha ($P < 0.001$), *H. floribunda* ($P = 0.002$), *M. oblongifolia* ($P = 0.005$), and annual Boraginaceae ($P = 0.005$) sites also experienced a significant difference in herbivory over the sample years. When *C. officinale* was pooled across sites, the mean percentage of herbivory per plant per subplot was about 31.8% in year one, about 29.4% in year two, and about 18.3% in year three (Figure 7, Table 2). Herbivory on *C. officinale* at the *H. micrantha* sites was about 29.3% in year one, increased slightly to about 32.3% in year two, and decreased to about 18.3% in year three (Figure 8, Table 2). Percentage herbivory on *C. officinale* at the *H. floribunda* sites was about 47.4% in year one, decreasing to about 30.6% in year two, and further decreasing in year three to about 13.1% (Figure 9, Table 2). The mean percentage herbivory of *C. officinale* at *M. oblongifolia* sites was about 13.0% in year one, increasing to about 34.9% in year two, and about 5.4% in year three (Figure 11, Table 2). Last, herbivory of *C. officinale* at the annual Boraginaceae sites in year one was about 42.4%, about 1.1% in year two, and increased to similar levels as year one with about 37.5% in year three (Figure 12, Table 2). The mean percentage of herbivory on *H. micrantha* in year one was about 20.7% per plant per subplot, in year two it increased to about 32.6%, and in year three the percentage of herbivory was like the previous year at 24.1% (Figure 8, Table 2). *Mertensia ciliata* herbivory was about 12.6% in year one, decreased to about 4.9% in year two, and greatly increased to about 41.2% in year three (Figure 10, Table 2). Percentage herbivory on the annual Boraginaceae followed a similar trend to *M. ciliata*; in year one herbivory was about 12.6%, about 5.5% in year two, and increased to 57.5% in year three (Figure 12, Table 2).

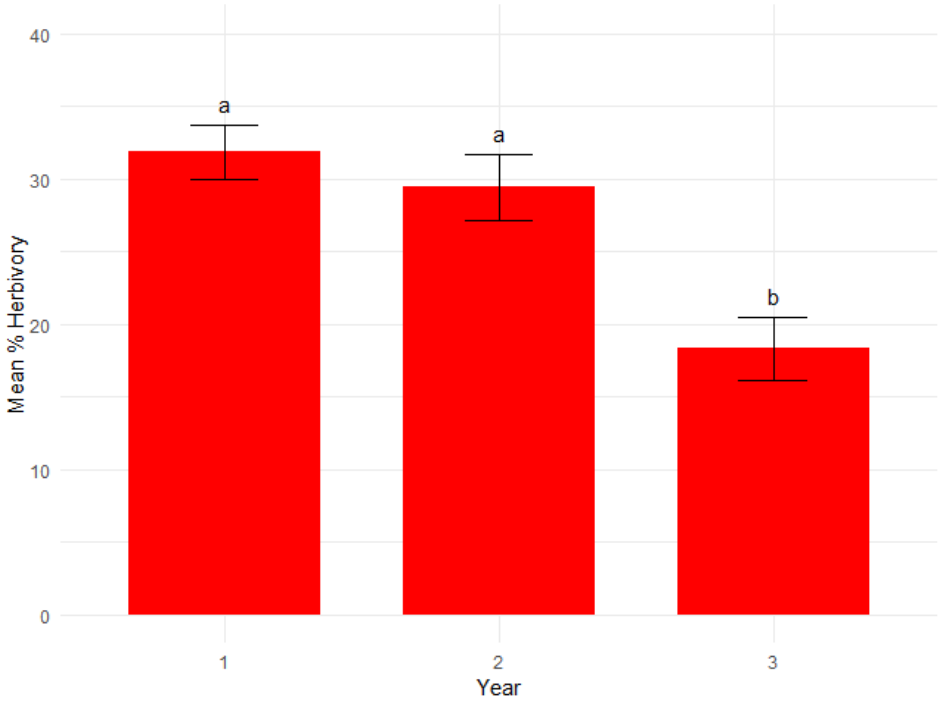

Figure 7. Mean percent herbivory per plant per subplot over the three sample years for pooled *Cynoglossum officinale* (all 14 sites). Error bars represent 1 standard error. Letters denote differences in means within a single species across years (α = 0.05).

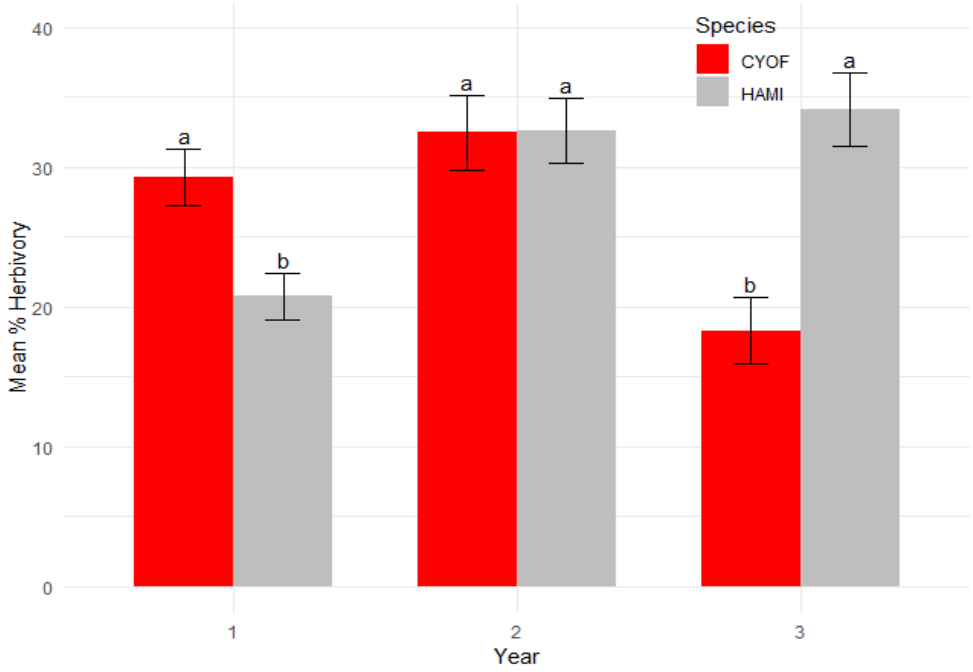

Figure 8. Mean percent herbivory per plant per subplot for *Cynoglossum officinale* (CYOF) and *Hackelia micrantha* (HAMI) over the three sample years at *H. micrantha* sites (14). Error bars represent 1 standard error. Letters denote differences in means within a single species across years (α = 0.05).

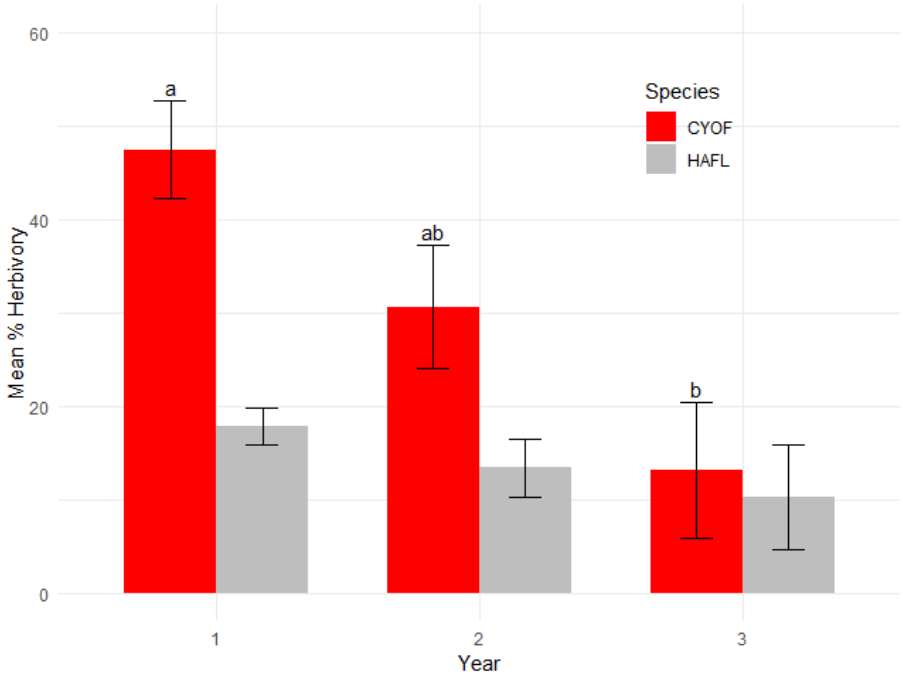

Figure 9. Mean percent herbivory per plant per subplot for *Cynoglossum officinale* (CYOF) and *Hackelia floribunda* (HAFL) over the three sample years at *H. floribunda* sites (2). Error bars represent 1 standard error. Letters denote differences in means within a single species across years ($\alpha = 0.05$).

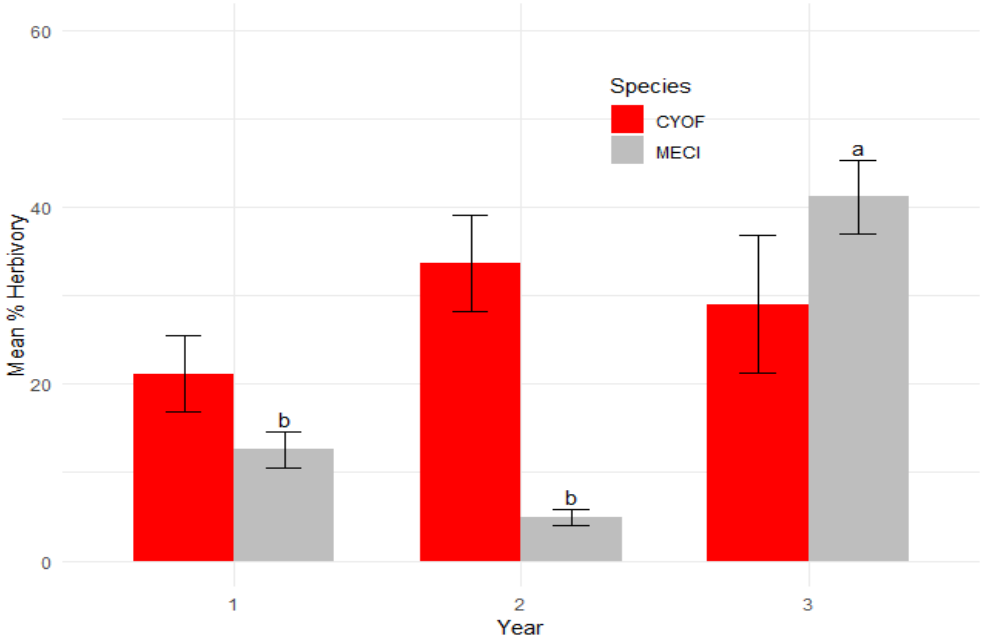

Figure 10. Mean percent herbivory per plant per subplot for *Cynoglossum officinale* (CYOF) and *Mertensia ciliata* (MECI)over the three sample years at *M. ciliata* sites (2). Error bars represent 1 standard error. Letters denote differences in means within a single species across years ($\alpha = 0.05$).

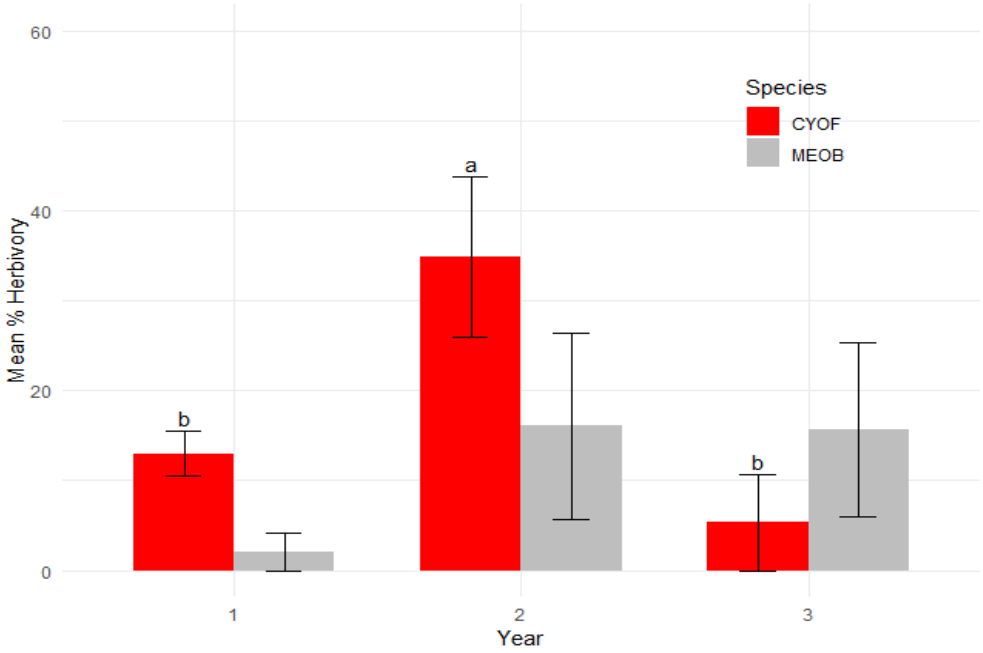

Figure 11. Mean percent herbivory per plant per subplot for *Cynoglossum officinale* (CYOF) and *Mertensia oblongifolia* (MEOB)over the three sample years the *M. oblongifolia* site. Error bars represent 1 standard error. Letters denote differences in means within a single species across years (α = 0.05).

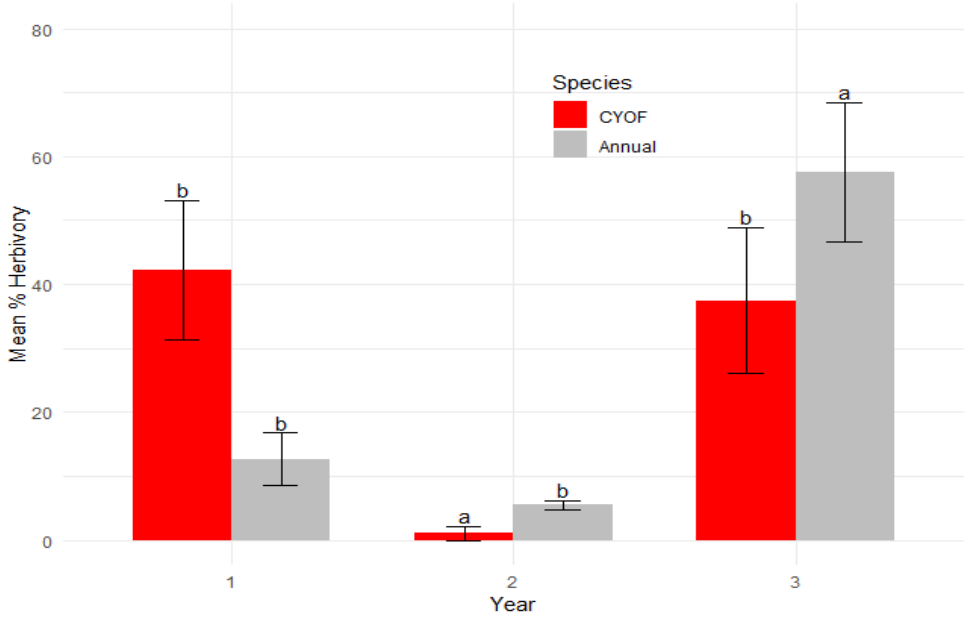

Figure 12. Mean percent herbivory per plant per subplot for *Cynoglossum officinale* (CYOF) and annual Boraginaceae (annual)over the three sample years the annual Boraginaceae site. Error bars represent 1 standard error. Letters denote differences in means within a single species across years (α = 0.05).

Relationship between plant counts and sample year, average oviposition, and percentage herbivory

The GLMM was used to evaluate if plant counts were influenced by sample year, average oviposition, and average herbivory for each non-target species separately and for *C. officinale* grouped by sites to match each non-target and pooled across all sites. Sample year negatively affected plant counts for two of the non-target species, *H. micrantha* and *H. floribunda*, as well as all but one of the groupings of *C. officinale* and the pooled *C. officinale* (Table 3). *Hackelia micrantha* plant counts decreased over the sample years with a coefficient of -0.184. In contrast, *C. officinale* plant counts at the *H. micrantha* sites decreased with a coefficient of -0.626 (Table 3). Also, *H. floribunda* plant counts decreased with a coefficient of -0.468 while *C. officinale* plant counts at the *H. floribunda* sites did not differ.

Hackelia floribunda plant counts were influenced by oviposition (Table 3). However, it was a positive effect with a coefficient of 0.506. The GLMM also indicated that there were multiple grouping analyzed where plant counts were influenced by herbivory (*H. micrantha*, *M. ciliata*, *C. officinale* at *H. micrantha* sites, *C. officinale* at *M. ciliata* sites, *C. officinale* at the annual Boraginaceae site, and pooled *C. officinale*) (Table 3). These relationships were modest, at best, with low positive coefficients (Table 3).

Table 3. P-values and coefficients from generalized linear mixed effects modelling (GLMM) of plant counts as affected by year, oviposition, and herbivory. Shaded and non-shaded bands inform the non-target (above) sites that *Cynoglossum officinale* results are associated with, pooled *C. officinale* does not have an associated non-target pair. The number of sites includes sites where the non-target species and target species was observed, and the number of observations includes the number of sub-plots summed across sites. Significant p-values are indicated in bold type ($\alpha = 0.05$).

Species	No. of Sites	No. of Observations	Year		Oviposition		Herbivory	
			Coefficient	P-value	Coefficient	P-value	Coefficient	P-value
Cynoglossum officinale (pooled)	14	600	-0.57	**<0.001**	-0.007	0.54	0.017	**<0.001**
Hackelia micrantha	10	453	-0.184	**<0.001**	-0.024	0.313	0.011	**<0.001**
C. officinale	10	468	-0.626	**<0.001**	0.005	0.737	0.016	**<0.001**
Hackelia floribunda	2	92	-0.468	**0.007**	0.506	**<0.001**	-0.006	0.536
C. officinale	2	57	-0.237	0.179	-0.054	0.093	0.006	0.197
Mertensia ciliata	2	111	-0.189	0.12	0.038	0.483	0.009	**0.044**
C. officinale	2	66	-0.846	**<0.001**	0.006	0.863	0.027	**<0.001**
Mertensia oblongifolia	1	20	-0.125	0.598	0.082	0.576	0	0.979
C. officinale	1	41	-1.745	**<0.001**	0.09	0.066	0.09	0.066
Annual Boraginaceae	1	13	-0.015	0.969	-0.218	0.43	-0.554	0.194
C. officinale	1	35	-0.556	**0.029**	-0.065	0.069	0.062	**<0.001**

Comparison of *Mogulones crucifer* juveniles developing in target and non-target plants

Mean number of *M. crucifer* juveniles (eggs and larvae) found during dissections was significantly different from *C. officinale* to all non-target species ($P < 0.001$, Table 4). Specifically, the mean number of *M. crucifer* juveniles found in *C. officinale* plants was about 14, which was higher than all non-targets; the number of juveniles found in all non-target plant species was similar and always less than 1.5 (Table 4).

Table 4. Mean number of *Mogulones crucifer* juveniles (larvae and eggs) ± 1 standard error found in different plant species totaled across sites. Significant p-values are indicated with different letters, denoting difference in mean *M. crucifer* juveniles developing in plant species. Letters denote differences in means of *M. crucifer* juveniles ($\alpha = 0.05$).

Species	No. Plants Dissected	No. Larvae	No. Eggs	Total No. Juveniles	Mean (±SE) Juvenile/Plant
Cynoglossum officinale	13	124	57	181	14.2 ± 5.5a
Hackelia micrantha	40	26	39	39	1.0 ± 0.3b
Hackelia floribunda	8	6	3	9	1.3 ± 0.5b
Mertensia ciliata	8	4	1	5	0.6 ± 0.5b
Mertensia oblongifolia	4	0	0	0	0.0 ± 0.0b
Annual Boraginaceae	3	0	0	0	0.0 ± 0.0b
Lithospermum ruderale	1	0	0	0	0.0 ± 0.0b

CHAPTER FOUR

DISCUSSION

The purpose of my study was to assess the interactions between the root weevil *M. crucifer* and its target host *C. officinale* and various non-target plants closely related to *C. officinale* in Montana and Washington. The results show that *M. crucifer* oviposited and fed on both *C. officinale* and non-target plants. My first hypothesis that *C. officinale* populations would decline more significantly than the non-target plant populations and the reductions would be correlated with increased levels of oviposition and herbivory, was partially supported. More consistent, significant declines of *C. officinale* populations were observed both when pooled across sites and when grouped to match non-target sites. However, the GLMM also indicated that *H. floribunda* experienced a statistically significant decrease in plant counts over the sample years, while *C. officinale* at the same sites did not. *Hackelia micrantha* also experienced a significant decrease in plant counts but *C. officinale* at the same sites experienced a more rapid decrease and to a larger degree. The same model also indicated that none of the observed, significant decreases in plant counts for non-targets or *C. officinale* groupings were significantly related to oviposition or herbivory, as all the coefficients associated with significant relationships with oviposition or herbivory were positive. Many plant species, whether they are native or invasive, can experience decreases in plant counts due to environmental factors (temperature, precipitation, elevation, slope, aspect, etc.) (Jansen et al., 2017; Pearson et al., 2017). I did not measure or include environmental variables in the model, but they could have contributed to the observed plant count decreases.

The difference in oviposition over the course of the study indicates that some non-target species, notably *H. micrantha* and *M. ciliata*, experienced significant levels of increased oviposition in one of the three sample years. However, the levels of oviposition experienced by the non-target species were lower than those experienced by *C. officinale* at the same sites in the same year. Additionally, these levels of increased oviposition on *H. micrantha* and *M. ciliata* could be related to the significant decrease in *C. officinale* counts in the same year. The decrease in the number of *C. officinale* plants at these sites could have potentially caused *M. crucifer* to increase its use of nearby non-target hosts for oviposition (Catton et al., 2015). Even with the significant increase of oviposition on *H. micrantha* and *M. ciliata* in year three, the same year dissections occurred, there were still more *M. crucifer* juveniles found developing in *C. officinale* compared to all other non-target species, suggesting that *C. officinale* is the preferred host. For example, I found nearly 14 times more juveniles in *C. officinale* plants than in non-target plants. This finding supported the second hypothesis that significantly more *M. crucifer* juveniles would be observed in dissected *C. officinale* plants than in dissected non-target plants. It is also consistent with the spillover that Catton et al. (2015) saw on *H. micrantha*.

Catton et al. (2015) found that oviposition scars were not reliable indicators of juvenile development in *H. micrantha*, with *C. officinale* plants with scars being three times as likely to contain developing *M. crucifer* juveniles as *H. micrantha* plants with scars. Potentially, *M. crucifer* is capable of ovipositing on non-targets, but the eggs are less capable of developing beyond this stage. Knowing if the ability to develop from egg to larva is reduced for non-targets is potentially very important information because the most detrimental damage inflicted on *C.*

officinale is during larval development, not through herbivory and oviposition (Prins et al., 1992). Therefore, if eggs are less capable of developing through subsequent stages, even if *M. crucifer* females are capable of ovipositing on non-target plants, this does not necessarily mean that those plants will experience significant or population-level impacts.

The steady decrease of *C. officinale* populations along with the consistent level of oviposition and higher occurrence of *M. crucifer* development within dissected plants, suggest that *M. crucifer* interactions are likely contributing to the suppression of its target host, which is consistent with research previously conducted (De Clerck-Floate et al., 2005; DeClerck-Floate & Wikeem, 2009; Catton et al., 2015). In general, the less consistent significant changes in non-target plant counts across sampling years in addition to the lack of significant, negative effect of *M. crucifer* oviposition and herbivory, and significantly lower incidence of observed *M. crucifer* juveniles in dissected plants, suggests limited population-level impact on these species from *M. crucifer* activity.

However, there are inconsistencies in the results of *M. crucifer* interactions correlating to plant reductions for the observed non-target species. Surprisingly, the GLMM indicated that oviposition and percent herbivory did not have a negative effect on plant counts for either the target or non-target species, while the ANOVAs suggested that mean percent herbivory and oviposition was significantly different and often increasing over the sample years. These inconsistencies suggest that further studies should be conducted to ensure that *M. crucifer* interactions are not leading to population-level impacts. My study was designed to gather data on a variety of non-target species to identify those experiencing negative effects from *M. crucifer*

interactions and may require additional more informative studies. For instance, continuing to observe sites for a minimum of five years, which would likely include years after *C. officinale* populations are nearly eradicated from the sites, could provide additional information regarding the nature of *M. crucifer* use of the non-target species (spillover vs sustained use) (De Clerck-Floate & Schwarzlander, 2002; Hinz et al., 2014). For non-target species that seem to be experiencing population declines where *M. crucifer* is also present, developing demographic models could be a valuable tool for evaluating actual population-level risks, which are not necessarily associated with signs of feeding or oviposition alone (Blossey et. al., 2018). My findings suggest that *H. floribunda* experienced a significant population decrease at two sites where *M. crucifer* was also present. However, my results further indicated that this population decrease was not related to *M. crucifer* oviposition or herbivory, but further investigation is warranted. De-Clerck-Floate and Schwarzlander (2002) also concluded that *H. floribunda* was a non-target species that should be monitored for negative impacts from *M. crucifer* after conducting host-specificity tests in Canada on non-target plant species than were not included on the original test-plant list. According to the Montana Natural Heritage Program (2024), *H. floribunda* has a state rank of S4, or "apparently secure" and a global rank of G5, or "secure." Regardless of these rankings, it is a good potential candidate for further demography studies.

CHAPTER FIVE

CONCLUSION

My study emphasizes the complexity and necessity of comprehensive, rigorous post-release monitoring for biocontrol agents, which is essential for validating pre-release, host-specificity predictions, ensuring that biocontrol agents like *M. crucifer* perform safely and effectively in the field. Given the expanding distribution of *M. crucifer* in the U.S. and its known oligophagous nature, supplementary intensive, species-specific monitoring is warranted for some of the non-target species where population-level impacts remain uncertain. Additional non-target species not included in this study should also be assessed to better determine all potential risks.

My findings support that *M. crucifer* contributes to the suppression of its preferred host *C. officinale*, but that continued exploration of potential non-target interactions and effects from *M. crucifer* are also warranted. Future work should focus on longer-term monitoring of non-target species, investigating additional non-target species, and using plant demography in cases where species are thought to potentially sustain population-level impacts related to *M. crucifer* interactions. Additional post-establishment evaluation of *M. crucifer* will inform the ecological safety and efficacy of this insect and its use as a biocontrol agent for *C. officinale.*

My study was designed to gather data on multiple non-target species that *M. crucifer* may have been interacting with, to determine if further, more detailed studies should be conducted. More rigorous species-specific studies are needed to decern the extent and effect of identified, concerning interactions. The monitoring protocol used in my study could be used in other areas

or for other non-target species that *M. crucifer* may be interacting with or to initially evaluate other biocontrol agent-non-target plant interactions. It provides a much needed first step in post-release monitoring to assess non-target plant use from biocontrol agents.

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