How Selective are Biological Controls?

Evaluating the potential for nontarget herbivory by *Mecinus janthinus* Germar [Coleoptera: Curculionidae], a biological control agent for Dalmatian (*Linaria dalmatica* L. P. Mill.) and yellow toadflax (*Linaria vulgaris* P. Mill.) [Scrophulariaceae]

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Thesis direct by Professor Timothy R. Seastedt

ABSTRACT

Invasions by introduced organisms are thought to have profound negative affects on biodiversity and ecosystem health and function. The continued expansion of two invasive plants, *Linaria dalmatica* and *Linaria vulgaris*, into natural areas throughout North America merits further investigation into the use of biological control agents. Introduced *Linaria* spp. have the potential to displace native plants, as well as alter ecosystems in which they inhabit. The most promising biocontrol agent currently available is *Mecinus janthinus*, a stem-boring weevil shown to stimulate significant declines in *Linaria* spp. populations. As with many biological control agents, concerns regarding nontarget effects have likely slowed the use of *Mecinus janthinus* for controlling invasive *Linaria* spp. This study employed three components to evaluate host specificity of *Mecinus janthinus*: greenhouse and field experiments of possible nontarget host acceptance, and release site observations of potential nontarget use where this weevil has established. Experimental choice and no-choice caged tests were conducted with *Mecinus janthinus* and a suite of Colorado native plants, were identified as those most likely to act as secondary hosts for this insect.

Both greenhouse and field experiments failed to demonstrate significant nontarget use of native plant species by *Mecinus janthinus* even when native plants were the only available hosts. These findings confirmed those of the pre-release host screening of this agent (Jeanneret and Schroeder 1992), however, host specificity tests alone may not accurately predict the use of alternative plant hosts in the field (Arnett and Louda 2002). For this reason, plant host use of *Mecinus janthinus* in the field following open release of the biocontrol was also assessed in this study. A total of 24 sites in Colorado and the Pacific Northwest were visited in the summer of 2004 where *Mecinus janthinus* has established on infestations of *Linaria dalmatica* and/or *Linaria vulgaris*. No evidence of nontarget use of native plants by *Mecinus janthinus* was found at any of these sites. Together these results support the continued use of *Mecinus janthinus* for the control of *Linaria dalmatica* and *Linaria vulgaris* based on a lack of evidence to support the potential for nontarget use by *Mecinus janthinus*.

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CONTENTS

APPENDICES

LIST OF TABLES

TABLE

LIST OF FIGURES

FIGURE

INTRODUCTION

Globalization has resulted in the transport of species around the world, both as deliberate and as unintentional introductions. Humans have removed many of the typical barriers associated with species invasion and have increased the movement of species overall (Lyons and Schwartz 2001). Among these transported species, some have been identified as having substantial negative economic and/or ecological impacts. Today, many are concerned about the profound consequences of invasive species on ecosystems, including the possibility of biodiversity loss and alteration of ecosystem services and function. Weedy species are no longer problems solely in developed, disturbed and cultivated areas; invaders are also finding their way into relatively pristine ecosystems. Invasive species are of particular concern in natural areas because such places often serve as the last refuges of local biodiversity where many important ecosystem services are performed (D'Antonio et al. 2004). Further, some exotic plants are purported to alter regional fire regimes, either by decreasing or promoting fire occurrence (Brooks et al. 2004). It is unlikely that expansion of invasive species will be curbed in the near future in light of the ever-expanding global economy of international trade, while global environmental change is expected to further exacerbate the spread of invasive species (Huenneke 1997).

Given the significant negative impacts associated with invasive species, land managers require means by which to control and/or eradicate invaders. Recent interest in the scientific mechanisms behind invasions has led to an increase in academic focus on the subject (D'Antonio et al. 2004; Seastedt and Luken 2004). Most would agree that each invader merits an individually devised control tactic, which renders the problem of invasive species even more complex. The approach advocated by Integrated Pest Management (IPM) prescribes that a variety of control methods be used to attack weedy species. Among the IPM methods considered promising is the use of biological control insects, or natural enemies. Biological control agents have the potential to significantly reduce pest populations with minimal cost, maintenance and toxicity (Hoddle 2004). As with many IPM tools, biological controls are not always effective nor without some risk of nontarget herbivory and indirect ecological effects (McEvoy 2002, Pearson and Callaway 2003).

This study focused on potential nontarget effects of control of two invasive plant species, Dalmatian toadflax (*Linaria dalmatica* (L). P. Mill) and yellow toadflax (*Linaria vulgaris* P. Mill.) [Scrophulariaceae], by the biological control insect *Mecinus janthinus* Germar [Coleoptera: Curculionidae]. Specifically, I tested for the possibility of nontarget herbivory by *Mecinus janthinus* on native plants found in the Colorado Front Range and the Southern Rocky Mountains. A three-fold approach was taken in order to evaluate host specificity: I used greenhouse experiments, field experiments and release site observations at locations with established populations of *Mecinus janthinus*.

BACKGROUND:

The Problem of Invasive Species

Invasive species are defined as those that have aggressively colonized a new region of the world by way of human introduction rather than by means of natural dispersal (Horvitz et al. 1998). Since the 15th century, worldwide movement of species has accelerated, as humans have intentionally and inadvertently distributed new plants, animals and other organisms to novel places. In the case of introduced plants, movement throughout the globe has largely occurred as agricultural introductions, as ornamentals, in animal waste, in ship ballast, or even as unnoticed hitchhikers on boats and other vehicles. It is thought that because introduced species did not co-evolve with a region's native community, some are able to out-compete natives via superior ability to sequester resources, escape from their own natural predators and pathogens, rapid growth rates, or ability to feed on organisms lacking coevolved defense and/or escape mechanisms (Davis 2003; Callaway and Ridenour 2004).

Characteristic traits of invasive species often include rapid growth rates and short reproductive cycles and many tend to be generalists that easily adapt to a variety of environmental conditions (Enserink 1999). The following are traits characteristic of many invasive plants: early maturation, profuse reproduction by seeds and/or vegetative structures, long-term seed viability, adaptations for spread via natural or anthropogenic means, allelopathy, animal or herbivore defense traits, ability to parasitize other plants, roots or rhizomes with large food reserves, survival and seed production under adverse environmental conditions, and high photosynthetic rates (Westbrooks 1998).

In addition to specific characteristics exhibited by non-native plant species, aspects of the physical environment may encourage successful colonization. Soil fertility and light availability likely act as important determinants of whether or not an introduced species will take hold (Wiser et al. 1998), while fluctuations in available resources may control ecosystem invasibility temporally and spatially (Davis et al. 2000). Resistance of an ecosystem to invasion by introduced species may also be a function of the strength of trophic interactions within a community (Lonsdale 1999).

Scientists agree that invasive species are profoundly altering ecosystems, both in terms of ecosystem function and species composition. These effects include direct threats to native species, changes in biodiversity, high costs to agriculture and negative human health consequences (Simberloff 2003). Attempts in recent years to quantify these changes have yielded mixed estimations of the true costs and consequences of invasive species. The former Secretary of the Interior, Bruce Babbitt, reported that exotic plants are invading wildlands in the United States at a rate of roughly 700,000 hectares per year (Pimentel et al. 2000). In 2000, it was estimated that economic losses caused by exotic species cost upwards of $137 billion annually in the United States alone (Pimentel et al. 2000). Invasive species were ranked second after habitat destruction among culprits in the threat to global biodiversity (Wilcove et al. 1998; but see also Gurevitch and Padilla 2004). However, little empirical evidence exists to support the contention that species extinctions have been directly caused by competition between native and introduced species (Davis 2003). Invasive species may be correlated with biodiversity loss and extinctions, but this is not necessarily a cause-effect relationship, as both are symptoms of habitat disturbance (Gurevitch and Padilla 2004). Even if invasive species rarely drive native species to extinction directly, they likely significantly reduce populations and act as contributing factors to the decline of sensitive species.

Global change factors are predicted to enhance environmental conditions that favor exotic plant species. For example, increased CO_2 in the atmosphere and amplification of nitrogen deposition may increase the ability of invasive species to colonize (Dukes and Mooney 1999). A positive growth response, via enhanced photosynthesis, is observed in many invasive species when grown in conditions of increased CO_2. Following Davis's (2000) theory of fluctuating resource availability, an increase in CO_2 could increase invasion opportunity by creating a surplus of available resources. Once established, non-native species may reduce resources available to native plants through competitive uptake (Dukes and Mooney 1999). Ultimately, distributions of invasive species will be modified, and likely expanded, by climate change factors (Dale et al. 2001).

Biological Control

Biological control can be an important tool in controlling invasive species and offers a promising alternative to other, more costly or more high-risk control methods. The use of biological control can reduce chemical use and mitigate corresponding side effects such as threats to human health and ecosystem integrity (Thomas and Willis 1998). In natural areas, biological control is a particularly attractive option for controlling weeds due to inaccessibility of terrain as well as a desire not to use chemicals on a large scale in such areas (McFadyen 1998; Mack et al. 2000; Hoddle 2004). Furthermore, monetary costs associated with the use of biological controls are negligible as compared to other methods of controlling exotic species (Hoddle 2004).

Classical biological control is defined as the control of invasive, introduced species with the introduction of natural enemies collected from the home range of an invasive species (McFadyen 1998). Biological control organisms act as top-down controls of a target pest, and when successful, exert a disproportionate effect on the abundance of the target organism. The enemy release hypothesis (ERH) is the theoretical foundation for biocontrol. In theory, the dynamics of biocontrol and target species interactions are similar to a predator-prey system (Pearson and Callaway 2003). Biological control theory predicts that agents will suppress target organisms below some threshold level, but will never completely eradicate target pests (Bellows 2001). Advantages of biological control include long-term suppression of a target invasive species, attack of a select number of species, few long-term monetary costs, and self-perpetuation of the control agent in most cases (USDA 2001).

Alien invaders tend to be larger and more vigorous in introduced ranges than in home distributions and this phenomenon has been attributed to more favorable environments in introduced ranges and to a lack of natural enemies (Blossey and Notzold 1995). Several names have been applied to the idea of liberation from specialist herbivores and pathogens in an invasive species home range, including the natural enemies hypothesis (Darwin 1859; Elton 1958), evolution of increased competitive ability (Blossey and Notzold 1995; Maron and Vila 2001) and the enemy release hypothesis (Keane and Crawley 2002). These hypotheses similarly predict that introduced species, through phenotypic plasticity, will eventually allocate fewer resources to defense from enemies and more resources to growth and reproduction (Blossey and Notzold 1995); thus allowing them to become superior competitors.

Successful management of invasive species with employment of biological control agents help to support the enemy release hypothesis (ERH), however this is not absolute evidence of ERH (Keane and Crawley 2002). To date there have been only a few direct tests of the theory (Maron and Vila 2001). One noteworthy study compared *Silene latifolia* Poir. [Caryophyllaceae], a perennial plant invader in North America, in the US and in Europe with its associated generalist and specialist enemies in each location. Populations in Europe were seventeen times more likely to exhibit pathogen and predator damage (Wolfe 2002). Lower levels of damage on North American *Silene latifolia* help explain the successful invasion of this plant and give support for ERH.

Other studies have suggested that an absence of fungal and viral pathogens and parasite enemies in new ranges similarly explain invasive species success (Klironomos 2002; Van der Putten 2002; Mitchell and Power 2003; Torchin et al. 2003; Torchin and Mitchell 2004). In an examination of negative and positive associations with soil microbes in home and introduced soil communities, Klironomos (2002) found that plants growing outside of native soil communities benefited overall. Plants grown in “exotic” soils were often free from soil pathogens, but were able to profit from general associations with mycorrhizal fungi. Geographic data compiled about plant associations with viruses and fungi yielded a strong correlation between lack of harmful viruses and/or fungi and invasion success (Mitchell and Power 2003). Introduced plant species were infected with fungi 84% less frequently and with viruses 24% less frequently than the same plants in their native range. Plant species that were more completely released from pathogen regulation were found to be more widely invasive.

Torchin and others (2003) tested whether a lack of parasites associated with introduced fauna helped explain their invasion success. In this examination of 26 invasive animals, twice the numbers of parasites were found on the organisms that were living in their native range as in those living in an introduced range. Torchin and Mitchell (2004) contend that parasites are important regulators of populations. They found that introduced plants escaped up to 90% of fungal and viral pathogens when transplanted to a new home. While introduced plants and animals do accumulate new enemies, it appears that the scale at which this occurs does not offset the benefit of escaping enemies at home (Torchin and Mitchell 2004).

Alternative explanations for success of invaders has been proposed, including the novel weapons hypothesis (Callaway and Ridenour 2004). Native plants are thought to be competitively disadvantaged by certain biochemicals released from introduced species (Callaway and Ridenour 2004). In an old versus new neighbors experiment, diffuse knapweed (*Centaurea diffusa* Lam.) [Asteraceae] from the Caucasus Mountains and from its new North American range was grown with similar coexisting grass species from each region in activated charcoal (Callaway and Aschehoug 2000). In these experiments, *C. diffusa* demonstrated a relative growth advantage over the North American grasses, which the authors attribute to the presence of an allelopathic chemical emitted by *C. diffusa*. The old neighbor grasses, meanwhile, produced more biomass as compared with the new neighbors, indicating that perhaps the Eurasian knapweed neighbors have evolved adaptations to cope with chemical substances produced by *C. diffusa*. A later study isolated the substance purported to be the active allelopathic chemical in *C. diffusa*, 8-hydroxyquinoline (Vivanco et al. 2004). The novel weapons hypothesis infers that regional scale adaptation to allelopathic chemicals occurring in native North American flora may be occurring as well as fluctuating geographic and regional coevolution (Thompson 1999; Callaway and Ridenour 2004).

Supporters of the novel weapons hypothesis claim that biological controls may just be ineffective in controlling invasive species with allelopathic compounds, since escape from natural enemies is not the mechanism responsible for their success (Callaway and Ridenour 2004). One study recorded a positive growth effect of spotted knapweed (*Centaurea maculosa* Lam.) [Asteraceae] when grown with one biocontrol insect, *Agapeta zoegana* [Lepidoptera: Cochylidae], introduced for the plant's control (Callaway et al. 1999). It was hypothesized that moderate levels of herbivory by *A. zoegana* actually stimulated growth of *C. maculosa* and induced allelopathic defense. Some evidence exists to support the claim that biological controls should be ineffective: 1) Some natural enemy biological controls are weak regulators of growth and survival of target species; 2) Negative effects by consumers may be the same in home and introduced ranges (Callaway and Ridenour 2004); 3) Consumer-plant interactions may not be as significant a control of ecosystems to begin with because, after all, the world is still green (Hairston et al. 1960).

Yet some biological control introductions have been extremely successful in controlling pest organisms and several examples follow. It is likely that a combination of mechanisms drive invasions, and those mechanisms that promote the success of invasive species may be unique to each species. If biological controls are expected to be successful, appropriate agents must be employed. Biocontrol insects must be capable of exerting a strong regulatory influence over the growth and reproduction of the host organism in the introduced range. Interaction strength in a plant-insect system and a biocontrol agent's escape from its own enemies at home are likely important in determining success of a potential biocontrol insect in a new range (Pearson and Callaway 2003).

Several examples of successful weed control by biological control exist and some will be highlighted here. In western Oregon, ragwort (*Senecio jacobaea* L.) [Asteraceae] was successfully controlled following the introduction of three biological control insects (McEvoy et al. 1991). At some sites, ragwort infestations were reduced to 1% of the original population. In the Front Range of Colorado, in Montana and in British Columbia, *Centaurea diffusa* has declined significantly following the introduction of several biocontrol agents (Seastedt et al. 2003; Myers 2004; Smith 2004). The success of biocontrol introductions in this case is attributed to significant reductions in *C. diffusa* seed production. In South Africa, biological control is considered a key management tool for controlling invasive plants (Moran et al. 2005). It is reported that 63 biocontrol agents have successfully established on 44 species of invasive plants in South Africa with 25% of those weedy plants completely controlled with biocontrol alone.

A wider analysis of biocontrol success indicates that approximately a quarter of all past biocontrol introductions are thought to have been effective (McEvoy 2002). This lack of overall success could be explained by a poor fit in biocontrol-target pest systems or by the fact that ERH may only partially describing invasion success. An overarching problem associated with the assessment of biocontrol and invasive species work is a lack of monitoring (Blossey 1999). Thus, success of biocontrol introductions is likely underreported, while nontarget effects could also go undetected. If land managers do not monitor biocontrol progress on invasive plant populations, several scenarios could result including a change to other control methods due to perceived lack of efficacy or alternatively, continued purchase and use of ineffective biocontrol agents. Another explanation for a lack of large-scale biocontrol success could be a lack of patience on the part of managers. Biological control should not be expected to reduce populations of target invasive species over the course of one or two seasons. Rather, implementation of biological control requires a time commitment of at least several years in most cases. However, once agents establish, long-term control can be achieved in at least some cases (McFadyen 1998).

Nontarget Herbivory and Biological Control

Although the release of biological control insects offers the potential for significant gains in invasive plant control, there are worthwhile concerns surrounding their use. Examples of nontarget herbivory (Louda and Potvin 1995; Louda et al. 1997; Hight et al. 2002) and lack of overall efficacy of biocontrol agents have prevented their widespread acceptance and implementation in many cases (Williamson and Fitter 1996). Examples of several pitfalls related to biocontrol follow.

Nontarget use of native species by biological control organisms has been documented in several systems. Critics of biological control claim that biocontrol agents are potentially more invasive than the invasive species they are introduced to control. The unintended exploitation of native organisms by biocontrols may pose a greater threat to native ecosystems than competition from invasive plants themselves (Louda et al. 2003b). In the case of vertebrate biological control introductions, there are several well-known examples of very destructive, nontarget effects associated with such introductions. These include the introduction of the small Indian mongoose (*Herpestes jananicus*) and the cane toad (*Bufo marinus*). The mongoose was introduced to islands in the Pacific and Caribbean to control rats; instead, native bird and reptile populations were decimated (Randall and Tu 2001). The cane toad was introduced throughout much of the tropics in the early 1900's to control agricultural pests, but subsequently had many negative effects on native fauna, including toxic effects on native tadpoles (Crossland 2000). Failures of vertebrate introductions in the past indicate that biocontrol introductions such as these unlikely in the future (McEvoy 1996). However, with regard to biological control insects for invasive plant control, specialist insects chosen as biological control agents often have more predictable and closer associations with a narrow range of plants (Herrera and Pellmyr 2002).

Several examples of nontarget herbivory in plant-biocontrol insect systems are worth further examination. *Rhinocyllus conicus* Frölich [Coleoptera: Curculionidae], a biological control weevil introduced in the late 1960's for control of musk thistle (*Carduus nutans* L.) [Asteraceae] was found to exhibit a host range beyond the target invasive plant in the field, which included some native plants. The insect significantly reduced seed production and successfully reproduced on several closely related native thistles including some rare species (Louda et al. 1997). The *Rhinocyllus conicus* case is often used as the poster child for why biocontrol may be dangerous. Preliminary host range tests of *Rhinocyllus conicus*, however, demonstrated that the weevil would, in fact, utilize native thistles, but was released despite this information (Rees 1982). At the time of this biocontrol agent's release, all thistles were considered undesirable. Thus, the *Rhinocyllus conicus* case should be viewed as a failure in judgment with regard to data interpretation rather than a failure to detect potential hosts by the pre-release host screening tests.

In another example of nontarget herbivory, the cactus moth *Cactoblastis cactorum* Berg. [Lepidoptera: Pyralidae] used for control of invasive prickly pear (*Opuntia* spp.) [Cactaceae], has been found to utilize native *Opuntia* spp., including threatened species, in the southeastern United States (Hight et al. 2002). The moth is expected to limit survivorship of young prickly pear plants including valuable crop varieties. This case of nontarget herbivory by a biological control agent differs from some cases since the cactus moth was accidentally introduced to Florida. The *Cactoblastis cactorum* case highlights pitfalls associated with the unforeseen effects that may occur when a biological control agent migrates.

The Eurasian weevil, *Larinus planus* F. [Coleoptera: Curculionidae], accidentally introduced in North America and later redistributed for the control of Canada thistle (*Cirsium arvense* (L.) Scop.) [Asteraceae] has recently been found to utilize and decrease the seed production of several native thistles in the Gunnison

Basin of Colorado (Louda and O'Brien 2002). Due to the higher elevation of the Gunnison Basin, the flowering time of the native Tracy's thistle (*Cirsium undulatum* (Nutt.) Spreng. var. *tracyi* (Rydb.) Welsh) [Asteraceae] more closely matched the life history of *Larinus planus*. Louda and O'Brien (2002) conclude that the establishment of biocontrol insects in "one ecoregion is not a scientifically sound basis for its distribution to other ecosystems…"

Along with the risk of nontarget herbivory by biological controls, these agents may have far-reaching, indirect effects on other trophic levels. Sometimes referred to as "ecological side effects" (Louda et al. 2003a), introductions of biocontrol agents may impact trophic interactions at other levels, even if a biocontrol agent is target host specific. A few examples of indirect effects exist, including instances of ecological replacement and altered food-web interactions (Pearson and Callaway 2003). One case cites a host specific biological control insect, *Urophora* spp. [Diptera: Tephritidae], responsible for providing food subsidies to deer mice because this fly does not control the target plant, *Centaurea maculosa*, and high densities of *Centaurea maculosa* support an abundant and unregulated *Urophora* spp. population (Pearson and Callaway 2003). The authors contend that increases in deer mice populations may result in higher incidences of Hanta virus. However, it is widely debated as to how widespread these phenomena of food-web alterations may be (Thomas et al. 2004). Indirect and direct risks associated with biological control should not be underestimated. Prediction of direct nontarget use may be achieved with the use of retrospective analyses of past nontarget cases (Pemberton 2000), while forecasting of indirect trophic nontarget risks likely requires complex food web models (Henneman and Memmott 2001) that are largely unavailable.

According to a review of 117 biological control introductions, of the 15 known cases of nontarget herbivory, biological control insects utilized congeneric plants in all but one instance (Pemberton 2000). Thus, “predictable risk” of nontarget herbivory by biological controls to native species most likely translates to closely related species. Specialist biological control insects introduced to suppress a target invasive species with few native relatives is less likely to impact nontarget natives, whereas, biocontrol introductions for target species with many closely related species is a more risky proposition. Another important consideration is how relationships among species are determined and used in pre-release testing of biocontrol agents. Older techniques of relating species based on morphological similarity may not have accurately determined true evolutionary relationships among species, and thus may not have predicted insect preference patterns on closely related plants.

Nontarget herbivory by biological control insects is a serious concern. However, it should be noted that many of the well-known cases of nontarget herbivory by biological controls have not occurred as a result of inadequate pre-release testing. Rather, nontarget herbivory cases have resulted either due to lack of judgment or due to accidental introduction. Many of these examples are not considered “true classical biocontrol”, and have thus lacked regulatory oversight. In the past, biocontrol agents with a somewhat broad diet may have been considered suitable for release if such an agent promised to significantly control a pest (Schaffner 2001; Hoddle 2004). There are no known examples of nontarget herbivory by intentionally released agents that did not exhibit similar behavior in pre-release screenings. Moreover, recent concern about nontarget herbivory has likely changed the criteria used to determine what constitutes a "good" agent and this awareness has improved pre-release screenings (Schaffner 2001; Pearson and Callaway 2003).

Efficacy of Biological Control

The multiple release strategy sometimes employed in biological control assumes that a greater number of agents may yield greater control. By employing multiple biocontrol agents that attack various parts of the pest plant at different life stages, control of pest plants through biological control may be more successful. For example, a suite of biocontrol insects for *Centaurea diffusa* have been shown to significantly reduce the abundance of the knapweed in Colorado (Seastedt et al. 2003). However, the idea that multiple releases are always superior presumes there is a positive interaction among the multiple agents and the combined affect of all agents is additive or synergistic rather than negative (Pearson and Callaway 2003). However, in more than 50% of the successful cases of invasive plant species controlled with multiple agents, a single agent is responsible for the success (Denoth et al. 2002). In fact, evidence suggests that a single insect species may be responsible for successful control of knapweed (Myers 2004; Seastedt et al. *in press.*). The multiple release strategy may be thought of as a "lottery," in which multiple agents are released with the intention of yielding at least one good biocontrol-pest match. The multiple release strategy is not always successful, nor desirable. The more numerous introduced biocontrol agents are, the greater the potential for negative, unintended results. In most cases, a single effective agent is preferable (Denoth et al. 2002)

In an experimental study of the relationship between establishment rate and the number of insects released it was, not surprisingly, found that a larger release of biocontrol insects translated to a higher chance of establishment (Grevstad 1999). The subsequent population growth rate of the introduced colonies also positively corresponded with initial release size. Grevstad (1999) examined the trajectories of two chrysomelid beetles *Galerucella calmariensis* and *G. pusilla* [Coleoptera: Chrysomelidae] used for the biological control of purple loosestrife (*Lythrum salicaria* L.) [Lythraceae] over a period of three years. Along with the finding that larger releases yield a greater probability of establishment, the importance of environmental influences and individual insect fecundities in the resulting population establishment is also highlighted. The significance of formulating a biocontrol release strategy (i.e., release of enough individuals to yield an established viable population) should not be understated.

Another factor that likely influences the effectiveness of a biological control is the role of plant quality. Insect feeding and performance (growth, survival and fecundity) may be significantly affected by plant characteristics such as plant architecture, nutrients and secondary chemicals. Plant quality may vary as a result of differences among genotypes (Underwood and Rausher 2000). Coevolution of plants and insects comes into play, as insects and specific plant genotypes may have tighter relationships than the same insect with other genotypes. An experimental study of four soybean (*Glycine max* (L). Merr.) genotypes and Mexican bean beetles *Epilachna varivestis* [Coleoptera: Coccinellidae] demonstrated that plant genotype can significantly affect the recruitment success and performance of insects

(Underwood and Rausher 2000). As applied to biological control, the insect-plant genotype match may affect the effectiveness of the biocontrol effort.

Introductions of non-native species, including invasive plants and biocontrols, may represent relationships outside of typical plant-animal interaction studies. Relatively rapid evolutionary changes may occur as introduced species interact with their new environments. Biological controls may represent novel plant-insect interactions (McEvoy 2002) and introduced biological control insects display the following traits that may differ from typical, well-studied plant-insect interactions: 1) biocontrol insects interact with invasive, exotic plants that are also behaving outside of normal conditions; 2) biocontrol insects, themselves, are imported; 3) biocontrol insects are released from their native natural enemies; 4) biocontrol insects tend to lack genetic variation; and 5) biocontrol insects and target plants are often found in disturbed environments.

Coevolution and Host-Switching

Although the biocontrol screening process addresses concerns regarding nontarget herbivory, it rarely addresses the possibility of host switching and coevolution over a longer evolutionary scale (Hoddle 2004). Coevolution is defined as a concordance between insect herbivores and plants over evolutionary time (Herrera and Pellmyr 2002). Ehrlich and Raven (1964) argued that a series of step-wise adaptations are responsible for coevolutionary relationships. Plants evolve physical and/or chemical defenses against natural enemies, and in turn, enemies evolve adaptations to cope with defenses. Often plant-insect interactions are described as an arms race between insects and plants (plant defenses versus insect adaptations to defenses).

The geographic mosaic theory of coevolution (Thompson 1994) predicts that ongoing, highly dynamic evolutionary processes define evolving species interactions across landscapes. Differential selection in distinct communities, termed selection mosaics, is proposed as an explanation for spatial and temporal variation in strength of relationships between species. Across geographic space, this theory identifies coevolutionary hot spots, where reciprocal selection occurs in some communities, as well as coevolutionary cold spots, where non-reciprocal section occurs in other communities. Coevolved traits of an interaction differ among communities and may demonstrate gradients or mosaics across the landscape (Thompson 1999). Thus, coevolution does not occur in all populations at all times across landscapes (Thompson 1994). Furthermore, the process of species coevolution may occur relatively quickly, at decadal time scales in some cases (Thompson 1999).

Coevolution, as it may apply to biological control systems, dictates that natural enemies of a particular invasive species may have a tight association with a given species in one locale and not in another. Therefore, it is important to consider the origin of both biological controls and the non-native target plant. Also, because it is hypothesized that rapid evolutionary change may be occurring in introduced species, biocontrol may be more effective in some areas than in others.

Several other evolutionary aspects of biocontrol should be considered, including genetic bottlenecks and resistance (aside from induced chemical defense) by target invasive species to biological controls (e.g., evolved plant resistance to herbicides).

Biocontrol introductions, as well as invasive species introductions, often produce genetic bottlenecks, which reduce genetic variability and alter selection trajectories (Hufbauer 2002). Some experimental evidence exists to support the claim that successful biocontrol becomes locally adapted in its new range. Also, lag times between establishment and population expansion of biological control insects may occur as a result of the time required for local adaptation, or microevolution. Although there are many documented cases of pesticide resistance, few, if any, examples of plant resistance to biological control insects exist. Biocontrol may be more evolutionarily stable than chemical control with respect to target organism resistance (Holt and Hochberg 1997). On the other hand, short generation times and large population sizes of biological controls may facilitate evolutionary adaptations in biocontrols and pests over relatively short periods of time, especially in response to environmental change.

With respect to host specificity of biological controls, some worry that although pre-release host specificity tests adequately predict host specificity of biocontrol agents over the short term, they may not predict actual host shifts over longer periods of time. It is thought that host switching in plant-phytophagous insect systems has been relatively common over evolutionary history (Percy et al. 2004). Based on phylogenetic evidence, patterns of host shifts have been more common between closely related plants than on more distant relatives (Janz and Nylin 1998). Phytophagous insects appear to adapt to changes in plant lineage by opportunistically colonizing new hosts (Percy et al. 2004). Plant secondary chemistry also plays an important role in evolutionary host shifts by insect herbivores. In analyses of historical patterns, strong correlations between host shifts and patterns of host chemical similarity have been demonstrated (Becerra 1997). Percy et al. (2004) write, "Plant-insect interactions are temporally, spatially and ecologically dynamic," thus ultimately predicting plant-insect interactions may prove difficult.

Returning to the well-cited *Rhinocyllus conicus* example of nontarget herbivory, Arnett and Louda (2002) conducted a re-test of the host specificity of this biocontrol agent. Pre-release tests demonstrated that *Rhinocyllus conicus* only marginally accepted most of the *Cirsium* spp. and strongly preferred *Carduus nutans*, the target plant host. It was hypothesized that host specificity had changed since original release in 1968, since the weevil was shown to utilize native thistles to a greater extent than originally predicted. However, results of the re-test (conducted more than 30 years after the release of *R. conicus*) indicated that host specificity had not, in fact, changed since the time of the original host specificity test, even after many generations and substantial numbers of the insect. Host-specificity tests of *Rhinocyllus conicus* had predicted acceptance, but did not predict magnitude of feeding and reproduction on native thistles in the field, the authors concluded. This result brings to light the notion that if a biological control is successful, it may reduce target plants to very low levels, in which case an acceptable, but less preferred, host plant might become relatively more vulnerable to nontarget use by a biocontrol.

The Biological Control Screening Process

The introduction of new biological control insects for weed control in the United States begins with a petition, by a researcher or other individual, to consider a specific agent for use as a biological control. A potential insect is observed in its home range, and if it exhibits a narrow diet, the Technical Advisory Group (TAG) accepts it for screening. TAG is a group within the US Department of Agriculture composed of representatives from federal agencies such as the Department of Defense, the Department of Interior, the Fish and Wildlife Service, and the Environmental Protection Agency. Agents under consideration are then tested for acceptance of nontarget plants (i.e., native plants, crop plants) in progressively more demanding tests. Following this screening and preceding any biological control release, an Environmental Impact Statement (EIS) and subsequent Finding of No Significant Impact (FONSI) are drafted. Elements such as geographical host range, known host range (host specificity), life history, distribution of target weed, potential impact on target weed and taxonomically related plants are included in the Environmental Assessment (EA) (USDA 2001).

ORGANISMS UNDER STUDY:

Linaria dalmatica, *Linaria vulgaris* and the Scrophulariaceae Family

Linaria dalmatica and *Linaria vulgaris*, both introduced from Eurasia, are common invaders in North America (Carpenter and Murray 1998). *Linaria dalmatica*, a yellow-flowered perennial, was originally planted in the US as an ornamental in the late 1800’s and is found throughout North America today. Mature plants grow to approximately 1-1.5 meters tall. Waxy gray leaves are broad and ovate in shape and clasp the stem of the plant. *Linaria vulgaris*, an attractive plant with yellow and orange snapdragon-type flowers, was also introduced to North America as a garden plant, probably in the mid-1600's (Volenberg and Krauth 1996). This plant is more common in the eastern United States, but is noxious in many western states as well.

Linaria vulgaris is similar in appearance to *Linaria dalmatica* but can be distinguished by its smaller overall stature and narrower leaves. *Linaria vulgaris* and *L. dalmatica* reproduce via prolific seed production and spread vegetatively by way of extensive horizontal root systems. A single *Linaria dalmatica* plant has been reported to produce on the order of a half million seeds (Grieshop and Nowierski 2002). Both species rely on insect pollination, usually bumble bees, and both are highly competitive with other plants, quickly colonizing sites disturbed by fire and grazing (Carpenter and Murray 1998). They are also known to invade relatively pristine ecosystems (Pauchard et al. 2003).

Due to the variable and, often, rugged nature of habitats invaded by both species of toadflax, management practices (e.g., mechanical, chemical and cultural) have yet to successfully control toadflax populations on a large scale. Control of *Linaria dalmatica* and *L. vulgaris* with chemicals is mixed, as these species have high genetic variability and a waxy coating on leaves that precludes effective penetration of herbicides (Vujnovic and Wein 1997; Lajeunesse 1999; Grieshop and Nowierski 2002). The Nature Conservancy states of these species, "permanent, long-term control cannot be achieved with herbicide treatment alone" (Carpenter and Murray 1998). Mechanical control efforts, including shallow tilling and hand pulling, are often unsuccessful due to deep taproots and extensive horizontal root systems.

Linaria dalmatica is thought to occupy 161,820 hectares in the United States (Duncan et al. 2004). A similar figure could not be found for *Linaria vulgaris*.

Several figures are available regionally for both species. In Colorado and the Rocky Mountains, several estimates of total area infested with *Linaria dalmatica* or *L. vulgaris* indicate that these species are widespread. However, the lack of consistency in these figures dictate that many more acres are likely impacted by these two invaders than is currently known. The State of Colorado estimates that *Linaria vulgaris* occupies at least 40,000 acres and *Linaria dalmatica* is estimated to occupy over 11,000 acres (Dept. of Agriculture 2002). The Nature Conservancy estimates that in the Southern Rocky Mountain Ecoregion (a portion of the Rocky Mountains extending from southwestern Wyoming to northern New Mexico), *Linaria vulgaris* and *L. dalmatica* occupy 41,048 acres and 11,366 acres, respectively (TNC 2004). In the Blanco District of the White River National Forest alone, *Linaria vulgaris* has been found on at least 20,000 acres, which includes significant portions of the Flattops Wilderness Area (H. Pearce, USFS, pers. comm.). The Blanco District has spent over $80,000 in the past three years in control efforts for this species.

In the Yellowstone ecosystem, *Linaria vulgaris* is considered to be among the most invasive plants (Pauchard et al. 2003). In the Rocky Mountains, pristine environments are considered at very high risk of invasion by *Linaria vulgaris* and resource managers at Rocky Mountain National Park (RMNP) consider this species to be the invasive plant of highest concern (J. Connor 2002, NPS, pers. comm.). In RMNP, *Linaria vulgaris* has been found growing in alpine environments far removed from its typical riparian habitat. A recent report states that *Linaria vulgaris* is a "significant threat to native biodiversity in open, human- or naturally disturbed environments in protected areas of the Rocky Mountains" (Pauchard et al. 2003).

Linaria dalmatica favors arid environments such as the Bitterbrush-Sage-Ponderosa Habitat, an ecosystem of conservation concern in Colorado (CNHP 2004) and can also be found in rocky, steep habitats. In Colorado, these species are both classified as "B List Species", meaning that the State is obligated to control the spread of these species (State of Colorado Effective May 2005). *Linaria vulgaris*, in particular, is listed as a species of special interest for control in Colorado.

The taxonomy and nomenclature of plants under consideration in this study will be discussed in some detail, due to the importance of taxonomic relationships in predicting use by biological control insects. New information about members of the traditionally circumscribed Scrophulariaceae family places some plant genera in families other than Scrophulariaceae (Olmstead et al. 2001; Albach et al. 2005). This finding indicates that a number of North American natives previously thought to be particularly in danger of non-target use by biological controls insects for *Linaria vulgaris* and *L. dalmatica* may not be as closely related as once thought.

Linaria vulgaris is commonly referred to as yellow toadflax or butter and eggs. *Linaria dalmatica* is called Dalmatian toadflax and synonyms of this species include *Linaria dalmatica* ssp. *dalmatica* (USDA 2004) and *Linaria genistifolia* (L.) P. Mill. ssp. *dalmatica* (L.) Maire & Petitm. (Weber and Wittmann 2001). Another species of invasive toadflax, *Linaria genistifolia* (L.) P. Mill. or thin-leaved toadflax, is easily confused with *Linaria dalmatica* but can be distinguished on the basis of thinner leaves and smaller flowers (Hitchcock and Cronquist 1973). It has also been reported that all three of these species hybridize, making positive identification difficult (Pauchard et al. 2003).

According to a review of non-target impacts by biological controls, if non-target herbivory is to occur in the case of toadflax biocontrol, insects are most likely to use plants in the same genus and, possibly, the same family (Pemberton 2000). The Scrophulariaceae family historically has been a large assemblage of genera distributed throughout temperate and tropical regions, whose members lack characteristic traits rather than commonalities (Olmstead et al. 2001). The reorganization of the Scrophulariaceae s.l. (sensu lato) into smaller, more distinct families (Olmstead et al. 2001) may make it easier to accurately predict likely secondary hosts. *Linaria* spp. belong in the Antirhinneae tribe considered part of the Scrophulariaceae family by many conventional references (Beidleman et al. 2000; Weber and Wittmann 2001; USDA 2004), but have been placed in the Veronicaceae (Olmstead et al. 2001) or Plantaginaceae (Albach et al. 2005) family in more recent treatments of this family. Penstemon have been placed in the Cheloneae within Plantaginaceae (Albach et al. 2005), while parasitic genera such as Castilleja and Pedicularis have been placed in the Orobanchaceae family. The phylogenetic placement of Mimulus is still in doubt, though it has been resolved in a clade with Lamiaceae (Olmstead et al. 2001). Many conventional sources, however, still consider these genera as part of the Scrophulariaceae. For simplicity, genera of interest in this study will be considered as belonging in the Scrophulariaceae family although as discussed previously, there is not widespread agreement.

The putative closest relative of the invasive *Linaria* spp. in the Rocky Mountain region is *Nuttallanthus texanus* (Scheele) D.A. Sutton (formerly named *Linaria canadensis* (L.) Chaz. var. *texana* (Scheele) Pennell) and is found in the same tribe, Antirrhineae (USDA 2004). This annual plant, commonly known as blue toadflax, is hypothesized here to be the most likely candidate for use by biocontrols for invasive toadflax. This species occurs at lower elevations along the Front Range in Colorado (Weber and Wittmann 2001). However, the diminutive overall stature, narrow stems and complete senescence of this plant at the end of the growing season may morphologically exclude extensive use by nontarget stem-boring biocontrol agents.

Nationwide, seventeen members of the Scrophulariaceae family are listed as federally threatened or endangered by the US Fish and Wildlife Service (USFS 2005). In Colorado, several species in the Scrophulariaceae are rare or imperiled, including at least a dozen *Penstemon* spp. and several *Mimulus* spp. (CNHP 2004). (See Appendix A.) Last year, the Nature Conservancy listed *Penstemon gibbensii* Dorn among the threatened species that declined the most in the Southern Rocky Mountain ecoregion (TNC 2004). This species is now ranked as a G1 species, critically imperiled globally, and was formerly considered a G3 species, globally vulnerable. *Penstemon penlandii* W.A. Weber, a federally listed endangered species (USFS 2005), is only known to occur in Colorado (USDA 2004). The significant number of imperiled species in the Scrophulariaceae family makes many land managers in the Rocky Mountains uneasy about the prospect of releasing biological control insects for toadflax control.

A group of secondary plant chemicals called iridoid glycosides occur in many members of the Scrophulariaceae family. It has been demonstrated that iridoid glycosides can be toxic, or serve as feeding deterrents to generalist insect herbivores

(Bowers 1991). Species in the tribe Antirrhineae are characterized by an iridoid glycoside, antirrinoside, which is thought to be nearly unique to this tribe (Ghebrehiwet et al. 2000). Although plant chemistry most likely plays an important role in the selection of host plants by *Mecinus janthinus*, this element was not directly tested in my research.

Mecinus janthinus, a biological control agent for *Linaria* spp.

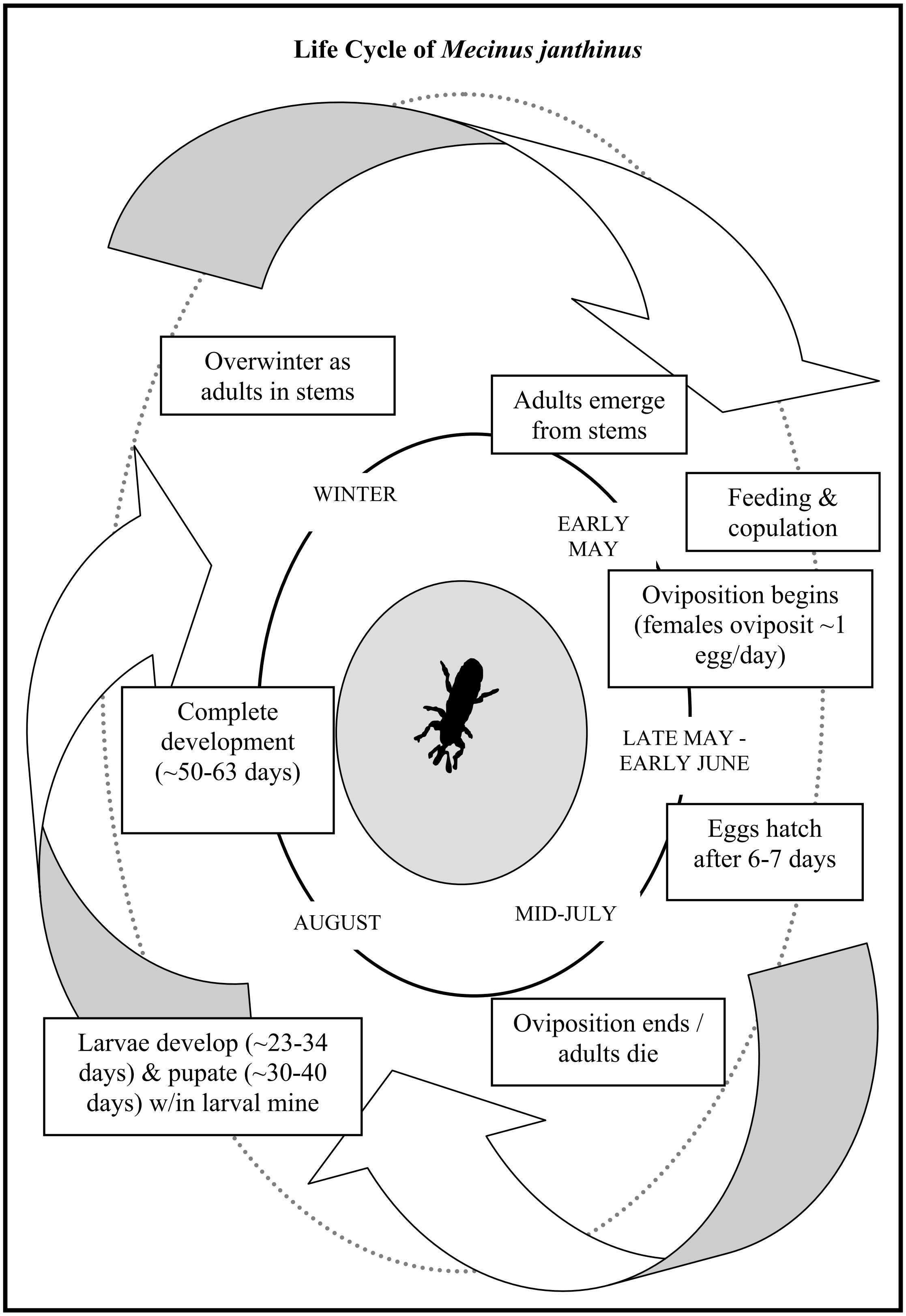
Mecinus janthinus is thought to be the most promising biological control agent currently available for the control of *Linaria dalmatica* and *Linaria vulgaris* in the United States and Canada (Hansen 2004). Native to Europe, this stem-boring weevil has been introduced throughout western North America. Adult feeding on plant growing tips and extensive tunneling within the stems by larvae significantly damages plants leading to mortality with repeated attacks (Hansen 2004). The damage caused by stem boring agents is expected to have a larger negative impact on the growth and spread of invasive toadflaxes than biological control insects that defoliate or feed on seeds. A Montana field study corroborated this prediction based on the finding that Dalmatian toadflax seedling recruitment seems to be more strongly controlled by plant competition than by seed limitation (Grieshop and Nowierski 2002). The home range of *Mecinus janthinus* encompasses a wide range of ecological conditions, as the insect is distributed throughout central and southern Europe, in a variety of climates and soil conditions (Nowierski 1994). As such, North American climates should be suitable for weevil survival in most places where toadflax is found.

Mecinus janthinus is a small black weevil measuring 3-5 mm in length (Hansen 2004). *Mecinus janthinus* requires a stem diameter of about 1mm in order for larvae to develop, limiting the potential host range of this insect to plants with substantial stem widths (Jeanneret and Schroeder 1992). For this reason, it is thought that *Linaria dalmatica* is favored over *Linaria vulgaris* by *Mecinus janthinus* because it has a substantially wider stem (Hansen and Gassman 2002). *Linaria vulgaris* also tends to emerge later in summer than *L. dalmatica*, which may also preempt a good match between *L. vulgaris* and *Mecinus janthinus*.

The life cycle of *Mecinus janthinus* spans one generation per year, the majority of which is spent in the stem of the host plant (Nowierski 1994). Adult weevils emerge from the stems in early May, depending on regional climate, to feed and copulate. During late May through mid July, females oviposit into small chewed cavities in the stem and then cover the opening with frass. Females usually lay one egg each day (Harris et al. 2003) and a single stem may receive up to 100 eggs over a period of many days (De Clerck-Floate and Miller 2002). Eggs hatch within approximately one week and larval development takes between 23 and 34 days (Nowierski 1994). Pupae form after 30-40 days, and overwinter within the plant stems until the following season (Fig. 1).

The results of a five-year Canadian study indicate that *Mecinus janthinus* is largely intolerant of sub-freezing temperatures (De Clerck-Floate and Miller 2002). Because overwintering occurs in above ground stems, the insects are particularly prone to mortality caused by cold temperatures and winter desiccation. Snow cover of stems can negate these effects to some extent. A temperature of –30º C is thought to

Figure 1. Life Cycle of *Mecinus janthinus.* Diagram by N. Breiter, data from Nowierski 1994



be a tolerance threshold, as mortality by *Mecinus janthinus* increased by an eight-fold margin at this temperature. However, despite high winter mortality in some areas, *Mecinus janthinus* has successfully established in cold climates.

The International Institute of Biological Control in Switzerland conducted host specificity tests of *Mecinus janthinus* using a range of potential host plants in 1988-1990 and subsequently recommended its distribution as a biological control agent in North America (Jeanneret and Schroeder 1992). These laboratory studies included adult feeding and oviposition tests and larval development tests with insects collected from southwestern Yugoslavia. Based on the Swiss group's favorable results, an Environmental Assessment recommended the use of *Mecinus janthinus* as a biological control in the United States (Nowierski 1994).

Mecinus janthinus was originally introduced as a biological control in Canada in 1991 (De Clerck-Floate and Miller 2002). It was approved for use in the United States in 1995 and first released in Montana in 1996 (Hansen 2004). Due to southern migration from the releases in Canada, the weevil was also found in Washington State, close to the Canadian border, in 1999 (L. Skillstad 2004, USDA, pers. comm.). However, large-scale distribution of this insect in the United States did not begin until the late 1990's, so widespread establishment of this insect has been limited in most locations. The State of Colorado began rearing this biological control in 1997 with subsequent field releases throughout the State beginning in 1998 (Jandreau 1999).

Several criticisms of the Swiss pre-release test, which are common to many pre-release biological control screenings, deserve consideration. First, these tests often focus on the vulnerability of crop and other economic plants to nontarget herbivory, while ignoring many native plants. In the *Mecinus janthinus* screening, only 8 of the 38 test plants used were native to North America. Only *Penstemon procerus* Douglas ex R. Graham var. *procerus* was tested for host acceptance by *Mecinus janthinus*. Commonly referred to as little penstemon, this plant is described as having a "slender stem" (Guennel 1995). The plant's morphology likely precludes reproduction by *Mecinus janthinus*. Also, cut shoots rather than live, potted plants were utilized in both the adult and larval feeding and reproduction tests. Lastly, and perhaps most critical, is the fact that pre-release host-specificity screenings often do not include field-testing. Thus, such tests are limited in their predictive power about how biological control insects will actually behave once released into a novel field environment (Schaffner 2001). These concerns merited a post-release investigation about the feeding and reproductive behavior of *Mecinus janthinus*, which is the focus of this study.

Hansen and Gassman (2002) conducted a subsequent host-specificity test to further examine potential use by *Mecinus janthinus* of native California *Antirrhinum* spp. not included in the Swiss tests. Although *Linaria* spp. were preferred, *Mecinus janthinus* used the *Antirrhinum* spp. in no-choice laboratory tests, indicating that these plants could be potential hosts. Field cage tests, however, yielded very little use of the *Antirrhinum* spp. (Hansen and Gassmann 2002).

Geographic Sources of Introductions

Invasive species and associated biological controls likely exhibit genetic variation based on differences in origin. As such, *Mecinus janthinus* individuals collected from different European sites could potentially demonstrate variation in host preference, based on principles of coevolutionary mosaic theory (Thompson 1999). Also, both *Linaria dalmatica* and *Linaria vulgaris* exhibit high genetic variability (Lajeunesse 1999). It is possible that if biocontrol agents were collected and introduced to North America, genotypic differences could translate to differences in efficacy or host preference (introduction of agents from new populations would, however, require new permits for international transport). It should be noted that all *Mecinus janthinus* individuals used in experiments in this study were received from US sources that are assumed to have originated from populations first released in the United States, and were thus most likely genetically similar (Table 1).

Table 1. Source of original introductions of organisms under study.

Organism	Use	Source	Date
Linaria dalmatica	Introduced to US as garden plant	?	1894[1]
Linaria vulgaris	Introduced to US as garden plant	Wales[1]	mid 1600's[1]
Mecinus janthinus	Pre-release laboratory screening by CABI	Southwestern Yugoslavia (Macedonia)[3]	1988-1990[3]
Mecinus janthinus	Release as biological control in Canada	Rhine Valley, France and Germany[4]	1991[5]
Mecinus janthinus	Release as biological control in US (Montana)	Rhine Valley, France and Germany[4]	1996[6]

[1] Information from (Carpenter and Murray 1998)
[2] Information from (Volenberg and Krauth 1996)
[3] Information from (Jeanneret and Schroeder 1992)
[4] Information from (A. Gassman 2005, CABI, pers. comm.)
[5] Information from (De Clerck-Floate and Miller 2002)
[6] Information from (Hansen 2004)

STUDY HYPOTHESES AND OBJECTIVES

Linaria dalmatica and *Linaria vulgaris* are invasive plant species with widespread distributions in the United States and Canada. Concern about the ability of these two species to colonize relatively undisturbed sites and natural areas have prompted the need for effective control methods. Ideally, control of these plant species can be achieved with minimal cost or maintenance, and no nontarget consequences. One biological control insect, *Mecinus janthinus*, has demonstrated promise in reducing populations of *Linaria dalmatica* and *Linaria vulgaris* in Canada (Hansen 2004) and is available for distribution in many Western States. In Colorado and the Southern Rocky Mountains, introduction of *Mecinus janthinus* is recent and the impact of this biocontrol in the region has yet to be seen. While *Linaria dalmatica* and *Linaria vulgaris* continue to expand into relatively pristine areas such as Rocky Mountain National Park, the Flattops Wilderness and the Yellowstone Ecosystem, employment of *Mecinus janthinus* to control these species in natural areas could result in mixed outcomes. While the biocontrol insect may significantly curb expansion of these invasive plant species, native plants may serve as secondary or even preferred hosts for this insect. Many land managers are reluctant to release biocontrol agents for *Linaria* spp. such as *Mecinus janthinus*, especially in light of the existence of closely related rare plant species found in this region that could be at risk of nontarget herbivory by such an agent (OSMP 2002).

The objective of this study was to expand on previous host preference testing of *Mecinus janthinus* by considering native species of the Colorado Front Range and Southern Rockies region specifically. This study included greenhouse and field experimental tests with *Mecinus janthinus* and a suite of native plants as well as field observations at sites where the insect has been released. I hypothesized that native plants in the Rocky Mountain region of greatest threat of direct nontarget effects by *Mecinus janthinus* would be either the most phylogenetically related species (i.e., *Nuttallanthus texanus*) or, alternatively, more distantly related plant species sharing similar morphological traits with *Linaria dalmatica* and *Linaria vulgaris* (i.e., *Penstemon* spp.). I also hypothesized that use of native plants in the field by *Mecinus janthinus* would be most likely in areas where the biocontrol insect has reached high densities and caused large-scale mortality of the target invasive species. The results of this study should be useful in the decision-making process of land managers considering the use of *Mecinus janthinus* for the control of *Linaria dalmatica* and *Linaria vulgaris.*

METHODS AND MATERIALS

Preliminary Analyses and Experiments

Preliminary Analyses

Prior to initiating greenhouse experiments, I conducted several preliminary statistical analyses using the *Mecinus janthinus* pre-release host screening data (Jeanneret and Schroeder 1992). A power analysis was employed to generate suitable sample sizes such that detection of statistically significant differences in insect use among plant species would be likely in my experiments (Zar 1999). Using the Jeanneret and Schroeder data (1992), I also made several statistical comparisons in order to determine whether *Mecinus janthinus* performance varied on *Linaria dalmatica* versus *Linaria vulgaris* and if there was a difference between insect preference on cut plant shoots versus live plants.

The results of the power analysis suggested that a sample size of 11 (power = 0.779) or 12 (power = 0.876) replicates would produce a detectable difference in insect use between the control plant and the native plants in no-choice tests. Analysis of feeding, oviposition scars and larvae variables on *Linaria vulgaris* versus *Linaria dalmatica* in the pre-release test demonstrated no significant difference among insect performance on the two species. Lastly, a Kruskal-Wallis test was employed to assess whether feeding by *Mecinus janthinus* varied significantly on cut plant shoots versus live potted plants of *Linaria vulgaris*. Mean feeding on potted plants was significantly greater ($X^2_{df = 1} = 4.3393$, $P = 0.0372$) than on cut shoots. As the use of live plants would also allow for insect observation over longer time periods than cut shoots, I determined that live plants would be used in all greenhouse experiments.

Preliminary Greenhouse Tests

A preliminary host acceptance test was conducted prior to initiating all other greenhouse experiments for the purpose of testing successful control plant (*Linaria dalmatica*) acceptance (feeding and oviposition) by *Mecinus janthinus* in the greenhouse. Live *Linaria dalmatica* plants were potted in 4-liter nursery containers and placed in enclosures (described below) with 4 weevils per enclosure. Feeding was scored weekly. Within 8 weeks, most adult weevils had ceased feeding and/or reproducing and had expired. Thus, it was established that an 8-week period would be sufficient to monitor feeding and reproductive behavior in subsequent experiments.

Greenhouse Experiments

All greenhouse experiments were conducted at the 30th Street Greenhouse, a facility owned and operated by the University of Colorado at Boulder. One room in the greenhouse was dedicated to this experiment; insecticides or other pesticides were not used in this room over the course of the experiments. The average daytime high was 30° C and average nighttime temperature low was 15° C. Relative humidity ranged from approximately 30-60% (greenhouse data from GROWLINK greenhouse detection system).

Materials

Enclosures were constructed with a cylindrical wire form placed over a plastic nursery container. The wire form and nursery container were then covered with remay fabric, a tightly woven, light- and moisture-permeable cloth. At seam edges, the remay fabric was folded over several times and the seams were stapled. Stapled edges were covered with duct tape and stapled again. The top of the cage was secured with clothespins. This configuration was used because it prevented weevil escape from cages, allowed light and moisture into the cages and prevented other insects from entering the enclosure. Enclosures measured approximately 15 cm diameter by 50 cm tall for 4-liter pots, 20 cm diameter by 70 cm tall for 8-liter nursery pots and 25 cm diameter by 90 cm tall for 12-liter nursery pots.

Plant material used in experiments was live and rooted in greenhouse potting mix (Fafard Mix #2 – 70% sphagnum peat plus perlite and vermiculite, manufactured by Conrad Fafard, Agawam, MA) in all cases. Plants were either grown from seed or salvaged as live plants from field sites. Seeds were hand-collected from Front Range sites or purchased from Western Native Seed Company and grown in the greenhouse. Plant salvage sites included the Left Hand Field Site (see site description) and Rocky Mountain National Park (See Appendix B). Plants were fertilized periodically with the greenhouse fertilizer-water injection system at a rate of 100 parts per million (Excel Fertilizer, 164ppm nitrogen - 33.5ppm magnesium - 50ppm calcium, manufactured by Scotts-Sierra Horticultural Products, Marysville, OH).

Mecinus janthinus individuals used for greenhouse experiments were obtained from several sources. In the preliminary host acceptance tests, treatment block 1 no-choice and treatment block 1 choice experiments, weevils were collected from field sites in the Front Range. In this case, uneclosed adults (somewhat active adult weevils not yet emerged from stem) were dissected from plant stems in early spring. Adult weevils used in the block 2 no-choice, block 2 choice and larval transfer experiments were collected by the USDA in the field as adults in northeastern Washington State (See Appendix B).

The sex of *Mecinus janthinus* individuals was determined using one of two methods. The simplest method involved the identification of pairs of mating weevils; male weevils mount the top of female weevils. In some cases a sufficient number of males and females could not be identified using this mating pair method, and weevils were then sexed under a dissecting microscope based on snout characteristics. Female weevils can be identified by their shiny and sparsely hairy snouts, while the male snouts are dull and hairy (R. Hansen 2004, USDA, pers. comm.).

No-choice Tests

No-choice tests, sometimes called "starvation tests," were conducted to determine potentially acceptable host plants to *Mecinus janthinus,* in a situation where no preferred host plants would be available. No-choice tests serve as the most vigorous of experimental tests of host acceptance (Schaffner 2001). All no-choice tests included a single live plant potted in a 4-liter nursery container and covered with an enclosure. Two female and two male weevils were placed in each enclosure. With the exception of two species, 14 replicates of each plant species were employed in the no-choice tests. Species used included the following: *Linaria dalmatica, Linaria vulgaris, Mimulus guttatus, Nuttalanthus texanus, Penstemon secundiflorus, Penstemon virens*, and *Penstemon virgatus.*

The no-choice tests were divided into two blocks of seven replicates per block. The blocks varied by timing and weevil source. Feeding was scored once per week for each enclosure for a period of 8 weeks. Adult feeding was ranked on a 0 to 3 scale with the following assigned values: 0 = no evidence of feeding, 1 = nibbling, 2 = frequent feeding marks, 3 = extensive feeding (adapted from Nowierski 1994).

Evidence of feeding by *Mecinus janthinus* is easily recognized by characteristic, small circular holes in leaves and flowers. After 8 weeks, enclosures were removed and the plants were analyzed for reproductive use by weevils. Oviposition scars were counted on each stem, as identified by a distinct brown circular indentation. Stems were then dissected and the number of larvae inside counted. In general, the number of oviposition scars exceeded the number of larvae in each stem. Thus, number of larvae may be a better indicator of survivorship and reproduction by weevils overall.

Choice Tests

Choice tests more closely simulate insect choice of a variety of potentially acceptable host plants in the field. In these experiments, the weevils were presented with a preferred host plant (*Linaria dalmatica,* in this case) and several alternative plants. Species used included: *Linaria dalmatica, Mimulus lewisii, Penstemon virgatus*, and *Castilleja* sp. Live plants were potted together in 12-liter nursery containers and covered with enclosures. Three pairs of weevils (3 males, 3 female) were placed in each enclosure. Twelve separate choice tests were conducted in total. Insect performance was assessed in the choice tests using the same methodology as in the no-choice tests: 8-week feeding observation period followed by quantification of oviposition scars and larvae in stems.

Linaria dalmatica/Linaria vulgaris Choice Tests

The relative preference of *Linaria dalmatica* versus *Linaria vulgaris* by *Mecinus janthinus* was also tested using choice tests. These tests employed 8 replicate pots containing both *Linaria* spp. planted in 8-liter nursery containers and covered with an enclosure. Two pairs of weevils (2 males, 2 females) were placed in each enclosure. Insect performance was assessed in the choice tests using the same methodology as in the other greenhouse tests: 8-week feeding observation period followed by quantification of oviposition scars and larvae in stems.

Larval Transfer Tests

Transfer of larvae from a preferred host plant to a native plant was conducted in order to assess potential survival and development of *Mecinus janthinus* larvae when placed into stems of alternative host plants. First, adult weevils were reared on *Linaria dalmatica* and allowed to oviposit. Once larvae developed in plant stems (ca. 30 days), larvae were dissected from plant stems with sterilized forceps and transferred into stems of a recipient transfer plant. Species used included: *Linaria dalmatica, Mimulus lewisii, Penstemon virgatus*, *Scrophularia macrantha*, *Veronica spicata, and Verbascum chiaxii* "Album". Small holes in the stems were artificially constructed on recipient plants and a single larva was placed inside each hole. The holes were then covered with a thin strip of tissue paper (Kimwipes EX-L produced by Kimberly-Clark Professional) to simulate the frass typically deposited on oviposition holes by the weevils. Stems were opened after 6 weeks and survival and development of larvae were assessed.

Data Analyses (Greenhouse Experiments)

All statistical analyses were performed using the SAS, Version 8 statistical analysis program (SAS Institute 1999). Confidence limits were set at 95% for all statistical tests. Several analyses were employed to test the following hypothesis as related to insect performance in greenhouse experiments:

- Null Hypothesis: Insect performance (feeding, oviposition, larvae) is not significantly different on the control plant species versus alternative host plant species.
- Alternative Hypothesis: Insect performance (feeding, oviposition, larvae) is significantly different (greater) on the control plant species versus alternative host plant species.

Since feeding data were collected as ranked scores, non-parametric statistical analyses were used to analyze this variable. The maximum feeding score was used instead of an average over 8 weeks in analyses because, in most cases, weevil feeding scores reached a maximum towards the end of the 8-week observation period. Oviposition and larvae data were analyzed with parametric tests, since these were count data.

No-choice Tests

A random block analysis of treatment block 1 versus treatment block 2 no-choice tests was conducted first in order to determine whether there were any differences among these treatment blocks (i.e., as a product of timing, weevil source). Interaction effects were also tested because results of a random block analysis can mask interaction effects (Gotelli and Ellison 2004). All subsequent analyses were conducted by pooling block 1 and block 2 no-choice data as a single data set. No-choice feeding, oviposition and larvae data of *Mecinus janthinus* on all plant species was analyzed using a Dunnett's test with *Linaria dalmatica* as the control, and *a priori* contrasts were used to make pairwise comparisons of insect performance between species.

Choice Tests

A Kruskal-Wallis non-parametric analysis of variance (ANOVA) was used to compare feeding scores in the choice tests. In a substantial portion of the choice tests, plants expired before the end of the 8-week observation, most likely due to interspecific competition, resulting in missing data points. Therefore, oviposition scars and larvae data were not statistically analyzed due to the large number of missing data points.

Linaria dalmatica/Linaria vulgaris Choice Tests

Mecinus janthinus performance on *Linaria dalmatica* versus *Linaria vulgaris* was compared by analyzing two datasets. First, weevil preference was directly tested between these two species in the *Linaria dalmatica/Linaria vulgaris* choice tests, in which the insects had both species available in a single enclosure. A Kruskal-Wallis analysis was employed to compare the maximum feeding scores for *Linaria dalmatica* versus *Linaria vulgaris* in the choice tests. Average feeding scores over the 8-week period were also compared using the same statistical test to determine if there was a difference in relative preference over time, as reflected more accurately by an average score. One-way ANOVA tests were used to compare oviposition and larvae on *Linaria dalmatica* versus *Linaria vulgaris* in the choice tests.

Insect performance on *Linaria dalmatica* versus *Linaria vulgaris* in the no-choice tests was also compared using a Kruskal-Wallis test (maximum feeding data) and a one-way ANOVA (oviposition and larvae data). The no-choice test data yielded information about *Mecinus janthinus* use of the two species independently.

Field Experiments

Left Hand Field Site Description

Field experiments were conducted at a privately owned 500-acre ranch in Left Hand Canyon, Boulder County, CO, USA. The site is located approximately 13 miles northwest of the City of Boulder (Fig. 2). The Left Hand Field Site is situated in a drainage with an ephemeral stream that flows into the larger Left Hand Creek. The drainage is oriented approximately west to east, with distinct north and south facing slopes rising on either side of the drainage. These slopes have distinct microclimates and differences in plant communities are readily apparent due to this topographic discrepancy.

This site offered an ideal setting for field studies involving invasive plants, due to a history of numerous disturbances that have allowed for the invasion by several exotic species. The land was historically used as a ranch, and cattle grazing is currently allowed on the lower segment of the property. A large percentage of the property has been burned during two recent wildfires. The Left Hand Canyon Fire burned through this area in 1988 and scorched much of the upper portion of the drainage. It is believed that heavy equipment used to suppress the 1988 wildfire introduced many invasive plant propagules to this site, which appeared following the burn. Presumably, north-facing slopes did not burn as hot, evidenced by many standing dead snags on these hillsides. In contrast, and most likely due to more intense fire conditions (as well as hotter and dryer southern slope conditions overall), tree and snag cover on southern aspects were sparse. In the fall of 2003, the wind-driven Overland Fire burned a majority of the south-facing aspects on the property.

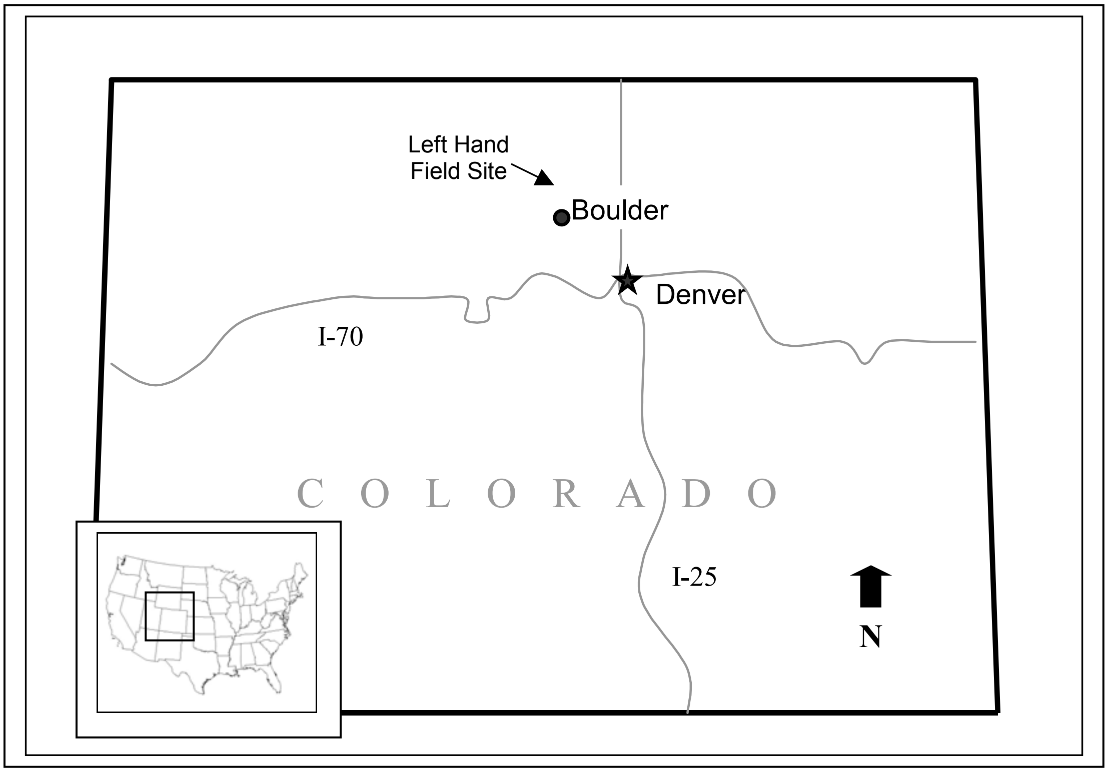


Figure 2. Study site Map, Colorado, USA. Map not to scale.

Some overstory tree mortality occurred, as well as large-scale mortality of tree seedlings. The following spring and summer of 2004, rainy conditions prompted significant soil runoff, particularly in streambeds. Another outcome of the 2003 wildfire was a visually significant increase in the density and spatial extent of *Linaria dalmatica* and other invasive plant species infestations at the study site (pers. obs.).

Vegetation at the site is characterized by pine savanna. A suite of native shrubs (such as *Acer glabrum* Torr.*, Jamesia americana* Torr. & Gray*, Oreobatus deliciosus* (James ex Torr.) Rydb. and *Rhus glabra* L.) and forbs (including *Geranium caespitosum* James, *Lithospermum multiflorum* Torr. ex Gray*,* and *Tradescantia occidentalis* (Britt.) Smith) are found beneath the more closed Ponderosa pine (*Pinus* *ponderosa* P.& C. Lawson var. *scopulorum* Engelm.) canopies. Where the canopy is more open or has been disturbed, several invasive plant species, including *Linaria dalmatica, Centaurea maculosa* Lam., *Potentilla recta* L., and *Carduus nutans* L., have established. Riparian areas tend to be densely vegetated by species such as *Betula fontinalis* Hook. and *Heracleum sphondylium* Bartr. Meadows are largely dominated by the introduced, invasive cheatgrass (*Bromus tectorum* L.), although some relics of the former mixed grass prairie exist including *Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths*, Hesperostipa comata* (Trin. & Rupr.) Barkworth*, Andropogon gerardii* Vitman and *Bouteloua curtipendula* (Michx.) Torr.

Mecinus janthinus was released to control *Linaria dalmatica* at the site in 2003 and again in 2004. The *Mecinus janthinus* individuals released in 2003 are presumed to have perished in the wildfire that same fall, since over wintering occurs in above-ground stems.

Field No-choice Tests

Field no-choice experiments were conducted at the Left Hand Field Site. Enclosure cages were installed around single *in situ* plants and 3 pairs of weevils were placed inside each cage. Ten replicates each of three different plant species were utilized. Each group of species used in the experiments, *Linaria dalmatica* (control), *Penstemon secundiflorus* Benth. and *Penstemon virens* Pennell ex Rydb., were located in distinct patches on south-facing mid-slopes. Specimens of each of these species were collected and deposited in the University of Colorado at Boulder Museum Herbarium (See Appendix C).

Enclosure cages used in field experiments were similar to those used in greenhouse experiments, but required fortification for field use. A small trough was dug around each plant with an approximate diameter of 30-45 cm and depth of 10-15 cm. Metal tomato cages covered with remay fabric were placed in each trough. The cages and remay were then buried at ground level. Cages were stabilized against wind with three 1 meter rebar stakes driven into the ground and attached to the cages with wire.

Weevils were placed in cages in early June of 2004 and insect feeding behavior was monitored every 2 weeks until the beginning of August. At the end of this 8-week period, stems were harvested and weevil reproduction was quantified. Oviposition scars on each stem were counted and stems were dissected in order to count larvae.

Release Site Observations

In order to evaluate host selection of *Mecinus janthinus* in the field, sites in Colorado and the Pacific Northwest were visited where this biocontrol has been released to control *Linaria dalmatica* and/or *Linaria vulgaris*. Colorado site information was obtained from the Colorado State Insectory. Release sites visited were selected on the basis of proximity to native plant communities. A total of 9 sites in Colorado were visited where *Mecinus janthinus* had been released in 2001 or later. These included sites on land belonging to the US Forest Service, Boulder Open Space and Mountain Parks, Jefferson County Open Space, and Lory State Park. For all sites, research permits or express permission to conduct research was obtained.

Due to the fact that few sites in Colorado could be located with large populations of *Mecinus janthinus*, sites in northeastern Washington State and northern Idaho with well-established weevil populations were also visited. Pacific Northwest site information was obtained via the USDA and several county agencies. *Mecinus janthinus* release sites in the Northwest and British Columbia are currently used for collection and redistribution throughout the US and Canada. Furthermore, the weevil has been found to disperse naturally to new toadflax infestations as well as to cause a major reduction in densities of large toadflax infestations there.

Methodology used to assess field behavior of the biocontrol was the same at Colorado and Northwest sites. At each location, the toadflax population and original *Mecinus janthinus* release site were located using GPS coordinates and release site maps. Stems and leaves of toadflax were evaluated for evidence of use (i.e., feeding, oviposition scars, larvae in stems or presence of adult weevils) by the weevil in order to positively identify existence of the biocontrol at each site. The spatial extent of the weevils within the toadflax infestation was then determined. Within and surrounding each toadflax patch, native plants in the Scrophulariaceae were identified, if any were present. Any visual evidence of feeding marks on flowers or leaves and oviposition scars on native plant stems was recorded. A subset of stems from native plants was then collected for later analysis. In the laboratory, stems were dissected in order to quantify the presence or absence and number of oviposition scars and larvae within stems (See Appendix D).

Plant specimens collected were deposited into the University of Colorado at Boulder Museum Herbarium (See Appendix C).

RESULTS

Greenhouse Experiments

No-choice Tests

The results of the random block analyses of insect performance between treatment block 1 and treatment block 2 indicate that there are significant differences between blocks for insect feeding and number of larvae. Differences between treatment blocks are common, however, and such differences can be adjusted for in analyses if necessary (Gotelli and Ellison 2004). A visual analysis of insect performance data by block indicates that these differences are proportional rather than interaction effects between blocks (Table 2 and Fig. 3). In tests of interaction between treatment block and species, larvae were the only factor with a significant interaction (Larvae: Block*Species $F_{df = 4} = 15.01$, $P < 0.001$, Oviposition: Block*Species $F_{df = 4} = 0.00$, $P = 1.00$, Feeding: Block*Species $F_{df = 4} = 0.93$, $P = 0.4535$). A graphical examination of the data (Fig. 3) demonstrates that this significant interaction effect is due to the large increase in larvae in *Linaria dalmatica* in treatment block 2, rather than the directional change in number among species. Subsequent statistical analyses of no-choice tests insect performance, discussed below, were conducted with treatment block 1 and treatment block 2 data pooled as a single data set.

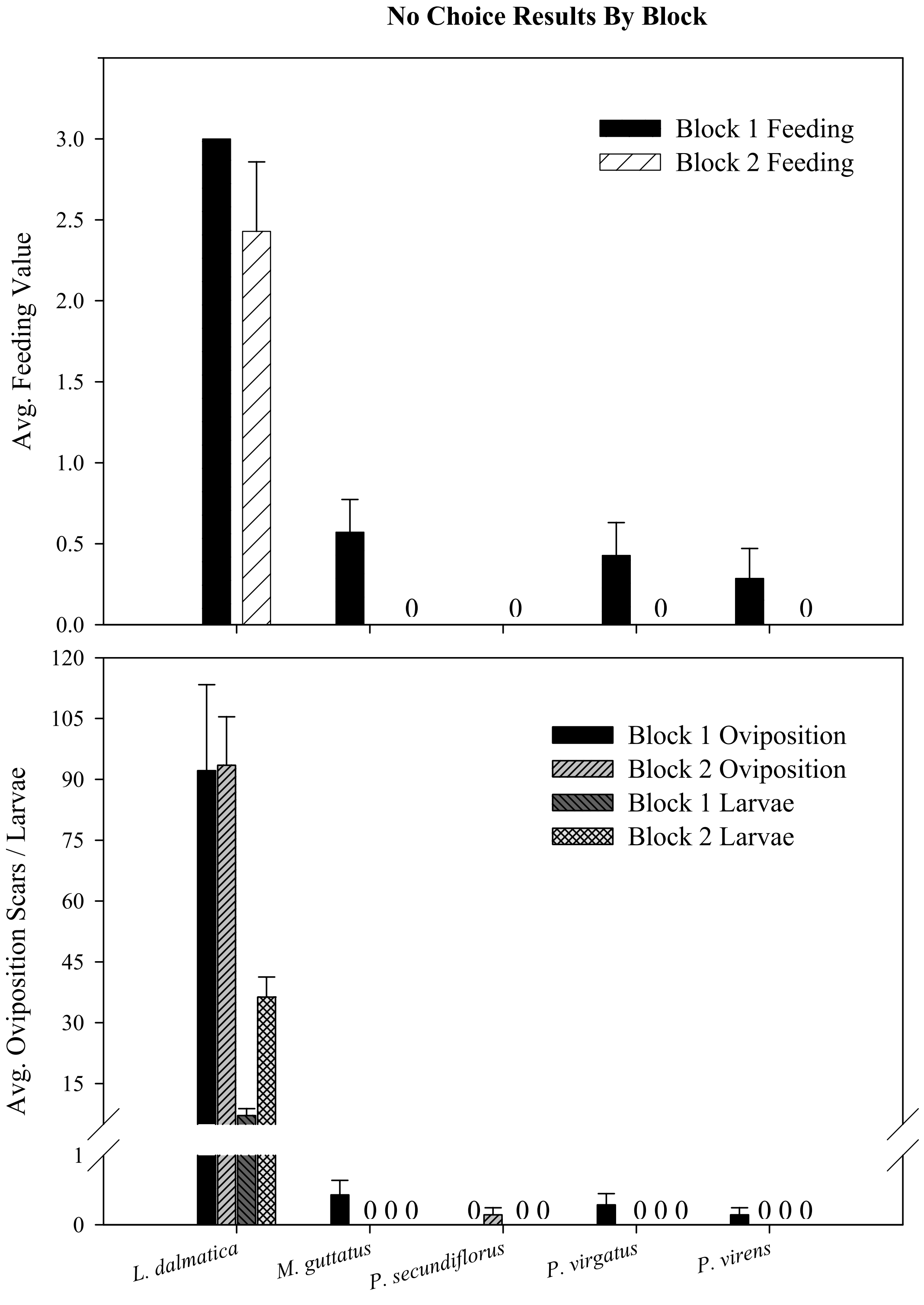
Table 2. Results of random block analysis

Dependant variable	DF	SS[1]	F value	P value
Feeding				
Block	1	2.23	9.80	0.0027
Species	4	71.49	78.56	< 0.0001
Oviposition Scars				
Block	1	0.39	0.00	0.9827
Species	4	88881.54	27.00	< 0.0001
Larvae				
Block	1	581.82	8.18	0.0059
Species	4	4396.56	45.46	< 0.0001

[1] Type 3 Sum of Squares

Note: *N. texanus* and *L. vulgaris* were excluded from block analysis because species were only present in treatment block 2 experiments

Figure 3. Results by treatment block. Error bars represent + 1 SE. N = 7 for all species per treatment block. Note y-axis break on second graph.

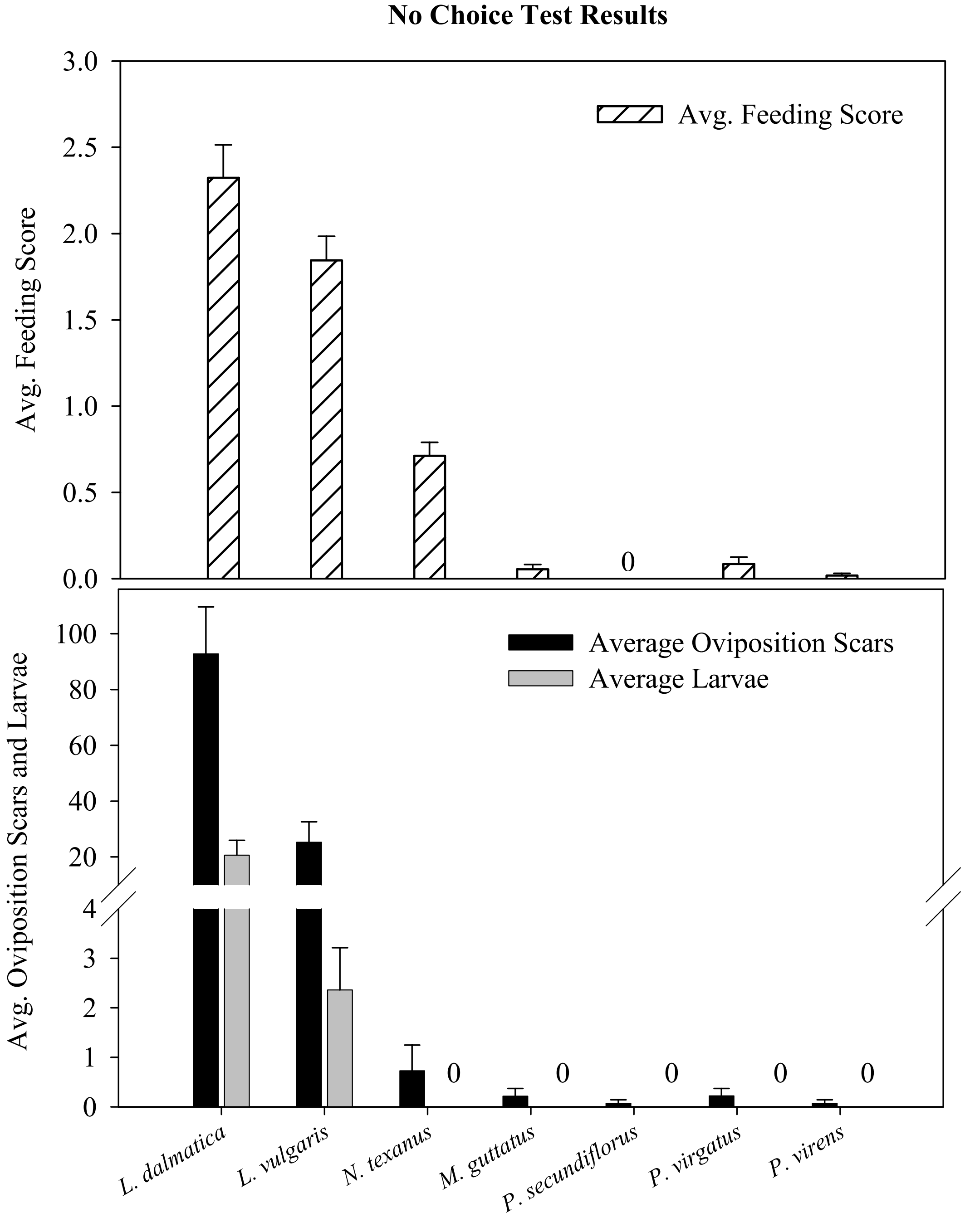


Feeding by *Mecinus janthinus* on *Linaria dalmatica* was significantly greater than that on all other plants in the no-choice tests was significantly greater ($F_{df = 1} = 138.21$, $P < 0.001$). *A priori* contrasts yielded a significant difference in feeding between *Linaria dalmatica* and all other species with one exception. There was no difference between feeding on *Linaria dalmatica* and *Linaria vulgaris*. *Mecinus janthinus* individuals oviposited in significantly greater ($F_{df = 1} = 119.10$, $P < 0.001$) numbers on *Linaria dalmatica* plants as compared to all other plants and *a priori* contrasts demonstrated this result in all pairwise comparisons. Number of larvae in stems was significantly greater ($F_{df = 1} = 68.12$, $P < 0.001$) in *Linaria dalmatica* plants than on all other plants; *a priori* contrasts revealed that this result was consistent in all pairwise comparisons (Table 3, Fig. 4 and See Appendix E).

Table 3. Summary statistics for no-choice tests.

Species	N	Mean Feeding Score	SE	Mean # Ovip. Scars	SE	Mean # Larvae	SE
Linaria dalmatica	14	2.71	0.22	92.77	17.49	20.62	5.49
Linaria vulgaris	14	2.57	0.20	35.40	8.24	3.30	1.07
Mimulus guttatus	14	0.29	0.13	0.21	0.15	0.21	0
Nuttalanthus texanus	12	1.42	0.15	0.73	0.54	0	0
Penstemon secundiflorus	14	0	0	0.07	0.07	0	0
Penstemon virens	14	0.14	0.10	0.07	0.07	0	0
Penstemon virgatus	9	0.44	0.18	0.22	0.15	0	0

Figure 4. Results of no-choice tests. Error bars represent + 1 SE. N = 14 for all species except N = 12 for *N. texanus* and N = 9 for *P. virgatus*. Note y-axis break on second graph.



Choice Tests

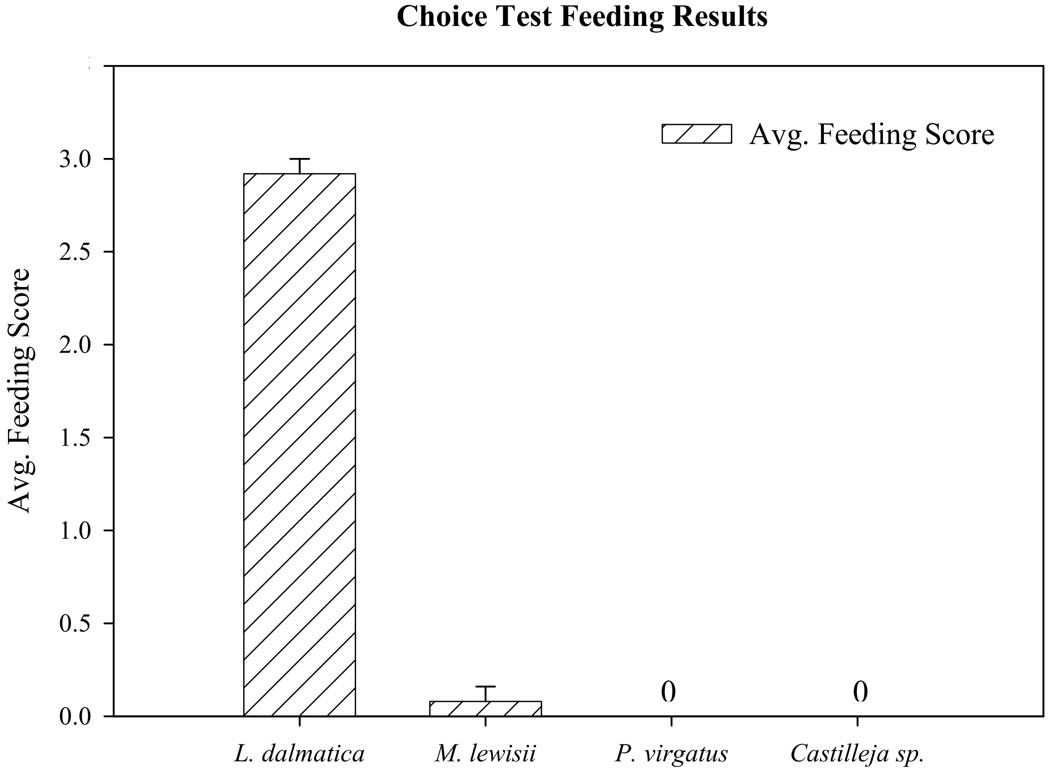
Mecinus janthinus feeding in the choice tests on *Linaria dalmatica* was significantly greater than on any of the alternate plant species ($X^2_{df = 3} = 40.45$, $P < 0.0005$). No analyses were conducted for reproductive data (oviposition scars and larvae), due to a high number of missing data points (Table 4, Fig. 5 and See Appendix E).

Table 4. Summary statistics for choice tests. N = 12 total choice tests

Species	N	Mean Feeding Score	SE
Linaria dalmatica	12	2.92	0.08
Mimulus lewisii	12	0.08	0.08
Penstemon virgatus[1]	12	0	0
Castilleja spp.	8	0	0

[1] *Penstemon secundiflorus* substituted in replicate 12.

Figure 5. Results of choice tests. Error bars represent + 1 SE. N = 12 total choice tests.



Linaria dalmatica/Linaria vulgaris Choice Tests

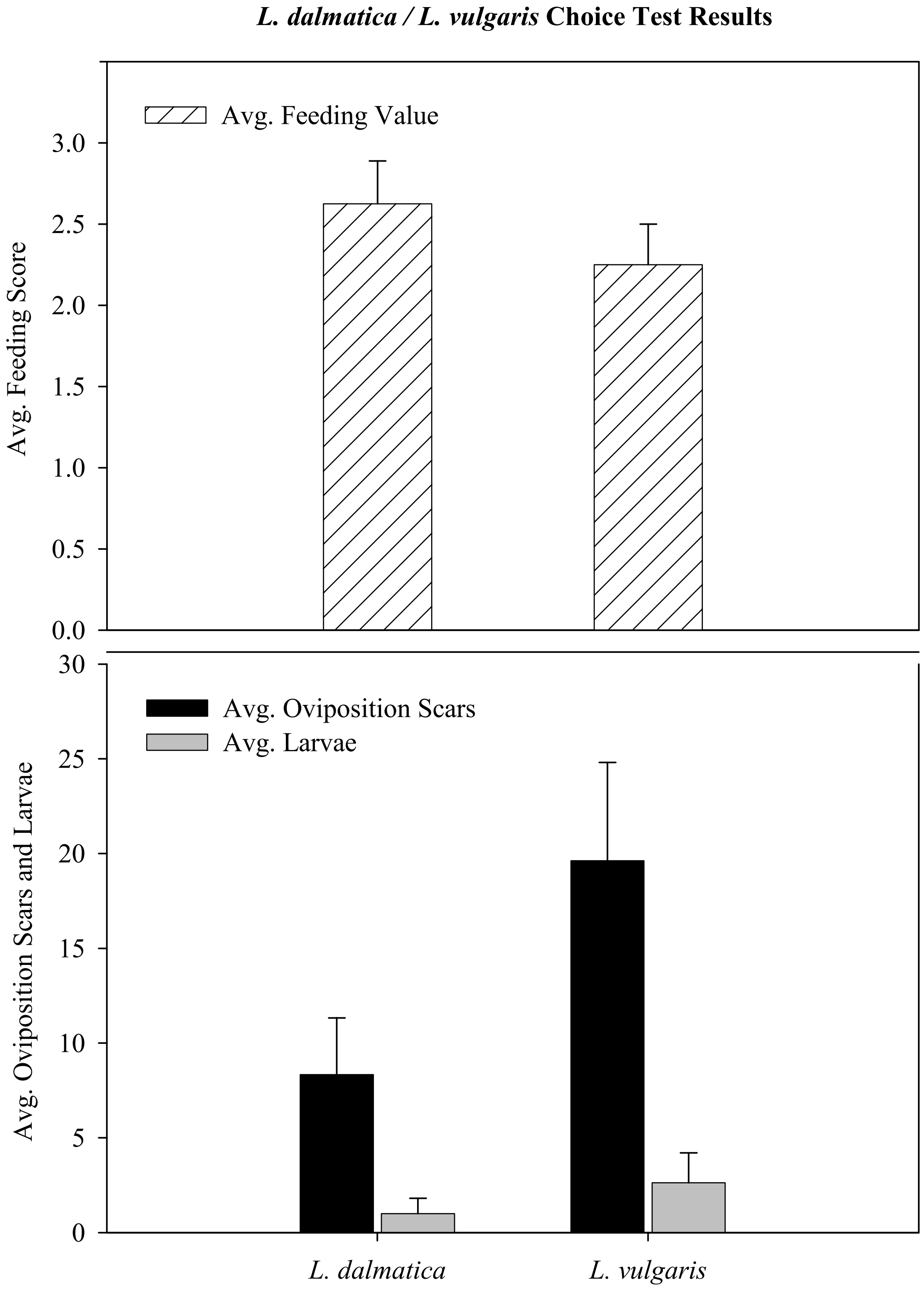
No significant difference was detected in maximum feeding scores in *Linaria dalmatica* versus *Linaria vulgaris* choice tests ($X^2_{df = 1} = 1.53$, $P = 0.2155$). However, when average feeding (mean over 8-week period instead of maximum value) was compared, average feeding on *Linaria dalmatica* in choice tests was significantly greater ($X^2_{df = 1} = 6.11$, $P = 0.0134$). Also, no significant differences were found in number of oviposition scars ($F_{df = 1, 12} = 2.96$, $P = 0.1113$) or larvae ($F_{df = 1, 12} = 0.68$, $P = 0.4258$) between the two plant species. A visual assessment of reproduction by the weevil on the two plant species reveals that values were greater on *Linaria vulgaris*. A simple comparison of proportion of oviposition scars to larvae in each plant species in no-choice tests indicates that eggs oviposited on *Linaria dalmatica* are more than twice as likely to reach the larval stage (22%) than those oviposited on *Linaria vulgaris* (9%) (Table 5, Fig. 6 and See Appendix E).

In a comparison of insect performance on *Linaria dalmatica* versus *Linaria vulgaris* in the no-choice tests (Table 3 and Fig. 4), results were similar to the choice test results with respect to feeding, but not reproduction. No significant difference in weevil maximum feeding values in the no-choice tests was found ($X^2_{df = 1} = 0.69$, $P = 0.4047$). The number of oviposition scars ($F_{df = 1, 12} = 7.26$, $P = 0.0136$) and number of larvae ($F_{df = 1, 12} = 7.40$, $P = 0.0128$) was significantly greater on *Linaria dalmatica* as compared to *Linaria vulgaris*.

Table 5. Summary statistics for *Linaria dalmatica/Linaria vulgaris* choice tests. N = 8.

Species	Mean Feeding Score	SE	Mean # Ovip. Scars	SE	Mean # Larvae	SE
Linaria dalmatica	2.63	0.26	8.33	3.00	1.0	0.82
Linaria vulgaris	2.25	0.25	19.63	5.19	2.63	1.58

Figure 6. Results of *Linaria dalmatica*/*Linaria vulgaris* choice tests. Error bars represent + 1 SE. N = 8 choice tests.



Larval Transfers

In all cases, including with the control plant, the larval transfer experiments were unsuccessful. Larvae did not survive or develop in any of the stems to which they were transferred. Most likely this can be attributed to unsuitable microclimate conditions in the greenhouse. No data analyses were performed.

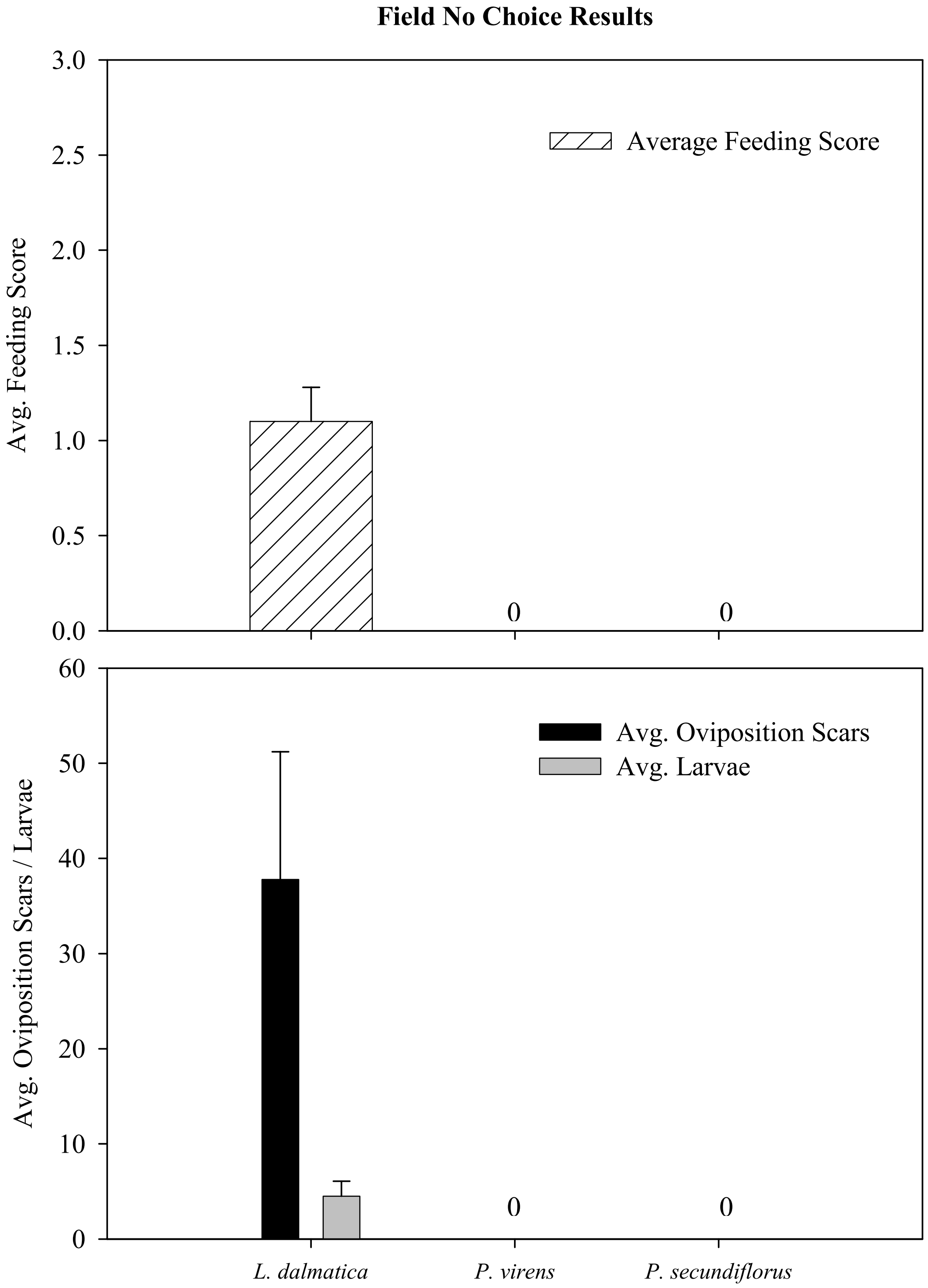
Field Experiments

The no-choice field cage experiments yielded limited data, due to unforeseen logistical difficulties. Some cages were damaged by the elements and insects were not recovered from these cages. Also, a few plants within enclosures died before the end of the observation period. Presumably, this mortality was caused by disturbance to the plant roots when burying the cages. Also, while enclosures appeared to successfully exclude most other insects, aphid populations (most likely present on plants when enclosures were placed) on several of the *Linaria dalmatica* plants increased substantially once the cages were constructed. Most likely, the exclusion of ants or aphid predators by placement of enclosures caused this result. Large aphid populations may have interfered with *Mecinus janthinus* behavior. Although feeding on the native *Penstemon* spp. by *Mecinus janthinus* was not observed, these results are inconclusive. Given these problems, no statistical analyses were conducted (Table 6, Fig. 7 and See Appendix E).

Table 6. No-choice field experiments results. N = 10 per species.

Species	Mean Feeding Score	SE	Mean # Ovip. Scars	SE	Mean # Larvae	SE
Linaria dalmatica	1.1	0.18	37.8	13.42	4.5	1.57
Penstemon secundiflorus	0	0	0	0	0	0
Penstemon virens	0	0	0	0	0	0

Figure 7. Results of field no-choice tests. Error bars represent + 1 SE. N = 10 per species.



Release Site Observations

The release site observations afford important results about the realized, or actual, niche of *Mecinus janthinus*. Of the 24 study sites visited where *Mecinus janthinus* had been released in Colorado and the Pacific Northwest, no evidence of nontarget herbivory was found at any of these sites. At 20 of 24 sites, established populations of *Mecinus janthinus* were found. At 10 of the 24 sites, populations of native plants in the Scrophulariaceae (*Penstemon* spp. and *Castilleja* spp.) were found growing either in or close to an infestation of invasive *Linaria* spp. with established populations of the biocontrol insect. In the field, no observations of feeding by *Mecinus janthinus* on leaves or flowers of the native plants were made Among the subset of those plants visually analyzed, a total of 169 native plant stems of three species were collected and dissected. No native plant stems dissected exhibited evidence of oviposition scars nor were larvae of the biocontrol insect found. As the table below summarizes, null values were entered for all feeding, oviposition and larvae variables measured on all plant stems collected (Table 7 and See Appendix F).

Table 7. Release site observations at sites with co-occurrence of *Mecinus janthinus* and native plants in the Scrophulariaceae. See Appendices D and F for more detailed information.

Site	Species	Stems collected	Feeding marks	Ovipos. scars	Larvae in stem
Site 2 – Mathew/ Winters Open Space Jefferson County, CO	*Penstemon secundiflorus*	40	0	0	0
Site 3 - White River National Forest, CO	*Castilleja* sp.	10	0	0	0
Site 4 - White River National Forest, CO	*Castilleja* sp.	8	0	0	0
Site 6 - White River National Forest, CO	*Castilleja* sp.	5	0	0	0
Site 9 – Rabbit Mountain, Boulder Open Space, CO	*Penstemon secundiflorus*	10	0	0	0
Site 18 – Railroad right-of-way near Kettle Falls, WA	*Penstemon procerus*	43	0	0	0
Site 19 – Abandoned airstrip near Northport, WA	*Penstemon procerus*	15	0	0	0
Site 20 – Abandoned orchard near Northport, WA	*Penstemon procerus*	15	0	0	0
Site 21 - Railroad right-of-way near Kettle Falls, WA	*Penstemon procerus*	9	0	0	0
Site 24 – Private field near Newport, WA	*Penstemon procerus*	14	0	0	0
Totals: 10 sites with natives	3 species	169 stems	0	0	0

DISCUSSION

The continued release of *Mecinus janthinus* as a biological control insect for invasive *Linaria* spp. is supported by the results of this study, on the basis of negligible secondary host plant use results. Studies of biocontrol host selectivity typically consider biological control agent introductions for very broad geographical regions while little post-release monitoring takes place. This study, however, specifically considered likely native host plants of *Mecinus janthinus* found in Colorado's Front Range and the Southern Rockies as well as included post-release assessments of this agent's host selection, or realized niche, in the field. Furthermore, while *Mecinus janthinus* has been considered a suitable agent for *Linaria dalmatica*, these results also offer encouraging evidence for *Mecinus janthinus* establishment on *Linaria vulgaris* in the field. The findings of this study do not serve as absolute evidence for the safety of *Mecinus janthinus* as a biocontrol agent, however, these results do substantially broaden what is currently known about the host selectivity of this agent.

Greenhouse Experiments

Several outcomes of the greenhouse experiments are worthy of discussion. In all cases any feeding marks, scars on plant stems resembling oviposition scars or larvae that appeared to be caused by *Mecinus janthinus* were recorded. Because these assessments were made on a visual basis only (i.e., larvae were not reared into adults), this technique may have inflated some feeding or reproduction scores, or even falsely identified use by the weevil on some plants. No-choice tests act as important simulations of field situations in which a biocontrol agent's range theoretically extends beyond the range of the target species, such that potentially acceptable nontarget species would be found in such areas. Thus, a lack of co-occurrence between the target species and an acceptable, secondary host species would create perhaps a greater potential for nontarget herbivory to occur. An example of this situation has been explored in several studies on *Rhinocyllus conicus* (Louda 1998; Louda et al. 2005). The co-occurrence of a target species and a secondary host species is simulated in choice tests. For this reason, the results of both no-choice and choice tests provide valuable insight into possible field host selectivity scenarios. Another important consideration to be extracted from these tests is whether a biological control insect can complete its developmental life cycle on a secondary, nontarget species. Results of this study indicate that *Mecinus janthinus* is unlikely to complete its life cycle on nontarget native species.

In the no-choice and choice tests, feeding by *Mecinus janthinus* on the alternate native host plants was observed at low levels as compared to feeding by the weevil on target species, *Linaria dalmatica* and *Linaria vulgaris*. Oviposition scars were recorded on several alternate plant species, but in all cases the mean number of oviposition scars was less than 1 per individual plant as compared with much higher numbers for *Linaria vulgaris* (mean = 35.40 scars) and *Linaria dalmatica* (mean = 92.77 scars). With the exception of one larvae found in the stem of a *Mimulus guttatus* individual (and thought to be *Mecinus janthinus* larvae, but not reared), no alternate host plants were found to successfully support this stage of reproduction by the weevil.

The only alternate native plant that consistently supported use by *Mecinus janthinus* in greenhouse tests was *Nuttalanthus texanus*. On other native plants, typically only a single feeding mark, oviposition scar, or larva of *Mecinus janthinus* was recorded, if at all. While feeding by the weevil on *Nuttalanthus texanus* in the no-choice tests did not damage plants enough to cause plant mortality, feeding was observed on all replicates of this species up to a maximum feeding score of 2 (frequent feeding marks). As many as 6 oviposition scars were found on a single plant, but no larvae were recorded on any of the *Nuttalanthus texanus* individuals. Also, *Mecinus janthinus* individuals failed to survive on *Nuttalanthus texanus* plants after week 3 of the 8-week observation period in the greenhouse tests in 9 out of 12 replicates. As originally predicted, this species appears to be the most acceptable alternative native host plant based on phylogenetic and, most likely, secondary chemistry similarities to invasive *Linaria* spp. Even so, this use appears to be insufficient to harm either individuals or populations of this species, unless feeding by adult weevils was great enough to cause significant decimation of seed production by such annual plant species. Use by *Mecinus janthinus* of other native plant species, such as *Penstemon* spp., hypothesized to support nontarget use based on similar morphology to *Linaria* spp., was negligible. These results indicate that nontarget use by *Mecinus janthinus* on this large and important genus is highly unlikely.

The life history and ecological attributes of *Nuttalanthus texanus* indicate that the likelihood of this species as a secondary nontarget host plant for *Mecinus janthinus* in the field questionable. Successful reproduction by *Mecinus janthinus* on *Nuttalanthus texanus* in the field seems unlikely due to the fact that this annual plant's stems are typically smaller than the width required for successful larval development of *Mecinus janthinus* inside plant stems (Nowierski 1994). Furthermore, because this plant is an annual or biennial and likely senesces completely at the end of the season, the above ground stems would not provide for overwintering by the weevil. Additionally, in the Rocky Mountain region, the distribution of *Nuttalanthus texanus* is limited to lower elevations (Weber and Wittmann 2001) such that the spatial overlap between this native species and the invasive *Linaria* spp. may not be common.

The lack of larval development by *Mecinus janthinus* on alternative native plants in greenhouse tests may have important implications for potential nontarget effects by this biocontrol. Larval stem mining, rather than feeding or plant seed reduction produces the majority of damage to plants caused by *Mecinus janthinus*. As such, if *Mecinus janthinus* fed on, but did not reproduce on nontarget plants in the field, damage would not be as extensive. Also, the impact of nontarget use by *Mecinus janthinus* would be expected to be greater on plant species whose expansion and/or reproduction is not seed limited, but rather controlled by other factors (Grieshop and Nowierski 2002). For this reason, because annual plants such as *Nuttalanthus texanus* are dependant on reproduction by seed, *Mecinus janthinus* may not be able to significantly limit this species even if it were to feed on this plant. In contrast, *Rhinocyllus conicus* is expected to substantially reduce populations of native thistles based on this agent's ability to limit seed production by species dependant on this form of reproduction (Louda et al. 1997; Louda 1998; Louda et al. 2005).

It is important to recognize that the ability of greenhouse tests to accurately predict host selectivity in the field has limitations. Greenhouse tests may not mimic the ecological conditions important in host selection by a biological control agent, such as phenological synchrony or other ecological factors not included in greenhouse tests (Schaffner 2001). Failures such as these by greenhouse tests may explain disproportionate use of nontarget species such as in the case of *Larinus planus*, the biocontrol inadvertently introduced and later redistributed for the control of Canada thistle (*Cirsium arvense*). This agent was found to use some native thistle species to a greater degree than predicted most likely due to plant life history traits that better match the requirements of *Larinus planus* (Louda and O'Brien 2002).

Performance on *Linaria vulgaris*

Another outcome worthy of further discussion is the performance of *Mecinus janthinus* on *Linaria vulgaris* in greenhouse tests and in the field. *Mecinus janthinus* is currently distributed for the control of *Linaria dalmatica* almost exclusively based on the current paradigm that these weevils may not establish and successfully control *Linaria vulgaris* due to this plant’s more diminutive stature (Hansen 2004). The results of this study suggest otherwise. *Mecinus janthinus* may, in fact, successfully establish on and potentially control *Linaria vulgaris*. In the greenhouse tests in which the weevil was given a choice between *Linaria dalmatica* and *Linaria vulgaris*, insect performance was not significantly different between the two species. In the no-choice greenhouse tests, there was no difference in feeding by *Mecinus janthinus* on *Linaria dalmatica* versus *Linaria vulgaris* but reproduction (oviposition scars and larvae) was greater on *Linaria dalmatica*.

In the field, two sites (one in Colorado and one in Washington State) were located where *Mecinus janthinus* successfully established (overwintered at least one year) on *Linaria vulgaris*. However, at neither site were *Mecinus janthinus* numbers large, or populations of the weevil widespread. The relatively good performance of *Mecinus janthinus* on *Linaria vulgaris* under greenhouse conditions contrasted with my observations of the weevil's lack of widespread establishment on this plant species in the field may be explained by environmental factors associated with the geographic distribution of *Linaria vulgaris* rather than traits specific to this plant. In the Rocky Mountain region, for example, *Linaria vulgaris* tends to occur at the higher elevations in the montane and subalpine zones as compared with the typical distribution of *Linaria dalmatica* in the foothills and montane zones. This difference in ecological distribution may have implications for the establishment of *Mecinus janthinus* on *Linaria vulgaris* in the Rocky Mountains. Higher elevations may translate to greater winter snowpack, which covers plant stems and protects overwintering adult weevils, and thus greater insulation. On the other hand, lower winter minimum temperatures at higher elevations could mean increased weevil mortality (De Clerck-Floate and Miller 2002). Another explanation for lack of *Mecinus janthinus* establishment on field populations of *Linaria vulgaris* could be the tendency of this species to emerge later in the summer than *Linaria dalmatica*, thus yielding a potential phenological asynchrony between the biocontrol and *Linaria vulgaris*.

Release Site Observations

Release site observations afforded a novel as well as applicable approach to evaluating the realized niche of *Mecinus janthinus*. It is rare that post-release monitoring occurs in the case of biological control introductions, however such inquiries have the potential to provide valuable information about both the efficacy and host selectivity of biocontrol agents once released (Simberloff and Stiling 1996; Thomas and Willis 1998). While introductions of biological control insects are not reversible (McEvoy and Coombs 2000), post-release information can be a useful tool for land managers when considering whether to continue introductions of biocontrol agents and whether or not agents should be released to new areas.

Many of the Colorado sites visited in this study exhibited poorly dispersed populations of *Mecinus janthinus*. At most sites, the weevils were only found within an average of 25 meters of the original release site. In contrast, sites in the Pacific Northwest tended to have well-dispersed populations of *Mecinus janthinus.* Most likely, this is a function of greater amount of time since introduction. The disparity in weevil establishment in the two regions could also be a result of greater winter snowpack in the Northwest and thus greater survival by the weevil in that region.

Where biological control insect populations become very large, or where biological control insects have caused the crash of a target invasive species the probability of nontarget herbivory increases due to an unmet requirement for host plants by the biological control. In this situation, insects must disperse, find alternative food sources or perish. Many sites visited with *Mecinus janthinus* in the Northwest exhibited either high insect numbers of *Mecinus janthinus* or actual crashes in toadflax populations (D. Palmer 2004, Ferry County, pers. comm.). For this reason, the lack of evidence of nontarget herbivory at these sites is a substantive and important finding that supports the claim that *Mecinus janthinus* is a host-specific biological control insect.

How does host selectivity of *M. janthinus* compare to other biocontrol agents?

It is useful to compare the *Rhinocyllus conicus* nontarget herbivory example to the predicted host specificity of *Mecinus janthinus*. In the pre-release tests of *Rhinocyllus conicus*, it was shown that several native species could serve as secondary hosts (Rees 1982). Thus, in the *Rhinocyllus conicus* case, nontarget effects in the field that have actually occurred, included significant use of several native thistle species and substantial seed reduction by the weevil (Louda et al. 1997; Arnett and Louda 2002) should have been predicted based on the pre-release test results. In comparison, both the results of my host specificity study and the pre-release test conducted with *Mecinus janthinus* (Jeanneret and Schroeder 1992) do not indicate use of native plants near the magnitude predicted by the *Rhinocyllus conicus* tests. Also useful in this comparison is the number of likely secondary hosts for *Rhinocyllus conicus* as compared to closely related and therefore likely alternate hosts for *Mecinus janthinus*. According to Pemberton (2000), likely secondary native plant hosts for biocontrols introduced for *Linaria* spp. are limited to three native plants (*Nuttallanthus* spp.) in the US, whereas closely-related native plants potentially affected by biological control insects for invasive *Cirsium* spp. number at least 90.

In the consideration of *Mecinus janthinus* as a suitable biological control, host specificity is conventionally defined as feeding and oviposition preference and larval development. However, host specificity alone may not necessarily predict use of native plants in the field, nor the magnitude of secondary alternate host use (Arnett and Louda 2002). Currently, there is healthy debate about how effective the pre-release host testing paradigm is at predicting the actual host behavior of an introduced biological control agent in a new range (Hoddle 2004; Louda et al. 2005). Observation in the home range of a candidate biocontrol agent and greenhouse studies alone are unlikely to provide absolute evidence for the safety of an agent in novel ecosystems. It has been proposed that not only does an agent's physiological host range need to be evaluated, but also its ecological host range (Louda et al. 2005). Greenhouse tests may predict secondary host acceptance or lack thereof, while simultaneously failing to provide reliable data on the magnitude of secondary host use in the field (Louda et al. 2005).

If *Mecinus janthinus* selection of alternative host plants is partitioned using the ecological criteria recently developed (Louda et al. 2005), then potential secondary host plants can be divided into two categories: 1) alternate host plants not acceptable to a biocontrol agent in a greenhouse setting and thus unlikely recipients of non-target herbivory in the field, and 2) alternative host plants accepted to some degree by a biocontrol agent in the greenhouse and translating to the possibility of nontarget use in the field. This comparison may serve as positive news relative to the host range of *Mecinus janthinus*. In greenhouse tests undertaken in this study, only one alternate native plant served as a mildly acceptable secondary host, *Nuttalanthus texanus*. However, as previously discussed, the degree to which this species may be exploited in the field remains unknown. In the Rocky Mountain region, large elevational gradients and a diversity of ecological microclimates are likely to translate to a range in relative phenologies and differences among life histories both for the biocontrol agent in question and target and nontarget plants under consideration.

Another consideration to be addressed, with respect to this agent and others, is that biological control may be the most effective and suitable means of managing weedy plants in natural areas (Mack et al. 2000; Hoddle 2004). However, natural areas offer the most significant collections of native plant biodiversity, translating to a high risk of biodiversity loss (Arnett and Louda 2002).

Conclusions

The risks and benefits of biological control introductions should be assessed on a case-by-case basis (Headrick and Goeden 2001). Not only should individual agents be evaluated using a risk-benefit framework, biocontrol agents should also be compared against other control methods used for invasive species as well as the no-action management alternative. Chemical control, for example, may be far less host-specific than biocontrol agents (Mack et al. 2000; Knight 2001). Invasions by exotic plants and their profound ecological consequences are predicted to continue and potentially intensify in coming years (Mack et al. 2000); thus effective and safe control methods are needed.

Post-release studies of established biocontrol agents, such as the one undertaken in this study, offer insight into what steps should be taken in advance of biocontrol releases in order to produce more effective and host-specific agents. Thomas and Willis (1998) wrote, “ Perhaps the greatest advances in improving our understanding of biocontrol could be made through increased post-release evaluation”. These authors suggest that studies of already released agents will provide needed data about agent efficacy, establishment, variations in host selectivity over time and space and trophic interactions at multiple levels (Thomas and Willis 1998).

Post-release studies can also be used comparatively to discover how adequately original host-specificity tests predicted host range. In a comparison between the results of this study and those found in the pre-release study conducted with *Mecinus janthinus* (Jeanneret and Schroeder 1992), we can ask whether modern testing procedures work. The agreement in results between the pre-release study and this study support the notion that current methods used for selection of biocontrol agents and subsequent host-screening activities are effective in screening-out potentially harmful agents while selecting agents with stricter host ranges than some biocontrol employed in the past. In conclusion, the results presented here support the continuing the use of *Mecinus janthinus* as a biological control agent for *Linaria* spp. based on a lack of evidence for nontarget herbivory by this agent. These results, too, serve as good news considering that this agent has already been distributed widely as a biological control.

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APPENDIX A. Imperiled species in the Scrophulariaceae family in the US and CO

US Threatened and Endangered Plants in the Scrophulariaceae Family

Latin Name/Common Name	Federal Status
Agalinis acuta Pennell Sandplain gerardia	E
Amphianthus pusillus Torr. Little amphianthus	T
Castilleja affinis Hook. & Arn. ssp. *neglecta* (Zeile) Chuang & Heckard Tiburon paintbrush	E
Castilleja campestris (Benth.) Chuang & Heckard ssp. *succulenta* (Hoover) Fleshy owl's-clover	T
Castilleja cinerea Gray Ash-grey paintbrush	T
Castilleja grisea Dunkle San Clemente Island indian paintbrush	E
Castilleja levisecta Greenm. Golden paintbrush	T
Castilleja mollis Pennell Soft-leaved paintbrush	E
Cordylanthus maritimus Nutt. ex Benth. *ssp. maritimus* Salt marsh bird's-beak	E
Cordylanthus mollis Gray *ssp. mollis* Soft bird's-beak	E
Cordylanthus palmatus (Ferris) J.F. Macbr. Palmate-bracted bird's beak	E
Cordylanthus tenuis Gray ssp. *capillaris* (Pennell) Chuang & Heckard Pennell's bird's-beak	E
Mimulus glabratus Kunth var. *michiganensis* (Pennell) Fassett Michigan monkey-flower	E
Pedicularis furbishiae S. Wats. Furbish lousewort	E
Penstemon haydenii S. Wats. Blowout penstemon	E
Penstemon penlandii W.A. Weber Penland beardtongue	E
Schwalbea americana L. American chaffseed	E

Source: US Fish and Wildlife Service
E = Federally-listed endangered species
T = Federally-listed threatened species

Colorado Rare Plants, Scrophulariaceae Family

Latin Name/Common Name	CNHP Rank	Federal Status
Penstemon breviculus (Keck) Nisbet & R.C. Jackson Little penstemon	G3/S2	---
Penstemon cyathophorus Rydberg Middle Park penstemon	G3G4/S2	S-BLM
Penstemon debilis O'Kane & J. Anderson Parachute penstemon	G1/S1	C
Penstemon degeneri Crosswhite Degener beardtongue	G2/S2	(3C),S-FS/BLM
Penstemon gibbensii Dorn Gibben's beardtongue	G1/S1	(C2),S-BLM
Penstemon grahamii Keck *ex* E. Graham Graham beardtongue	G2/S2	C,S-BLM
Penstemon harringtonii Penland Harrington beardtongue	G3/S3	(C2),S-FS/BLM
Penstemon parviflorus Pennell Small-flower beardtongue	GX/SX	(C2*)
Penstemon penlandii W.A. Weber Penland beardtongue	G1/S1	E
Penstemon retrorsus Payson ex Pennell Adobe beardtongue	G3/S3	C2
Penstemon scariosus Pennell var. *albifluvis* (England) N. Holmgren White River penstemon	G4T1/S1	C,S-BLM
Penstemon scariosus Pennell var. *cyanomontanus* Nesse Plateau penstemon	G4T?/S2S3	---
Mimulus eastwoodiae Rydb. Eastwood monkey-flower	G3/S1S2	S-BLM
Mimulus gemmiparus W.A. Weber Weber monkey-flower	G2/S2	(C2),S-FS

Source: Colorado Natural Heritage Program (CNHP)
Explanation of ranking can be found at the following website:
http://www.cnhp.colostate.edu/ftp_meta/element_2003.pdf

APPENDIX B. Source of plant and insect materials used in greenhouse experiments

Experiment	Plant	Block	Source	#	Insect	Source	Stage	#
Preliminary Host Acceptance Test	*Linaria dalmatica*	n/a	University of Colorado (east campus) - Vegetative and seed	10	*Mecinus janthinus*	Front Range Field Collection Site, CO	Uneclosed Adults	4 per replicate
No Choice Test	*Linaria dalmatica*	1	University of Colorado (east campus) - Seed	7	*Mecinus janthinus*	Front Range Field Collection Site, CO	Uneclosed Adults	4 per replicate
No Choice Test	*Linaria dalmatica*	2	University of Colorado (east campus) - Seed	7	*Mecinus janthinus*	NE Washington Collection Site via USDA	Eclosed Adults	4 per replicate
No Choice Test	*Linaria vulgaris*	2	Rocky Mountain National Park - Salvage	14	*Mecinus janthinus*	NE Washington Collection Site via USDA	Eclosed Adults	4 per replicate
No Choice Test	*Penstemon virgatus*	1	Rocky Mountain National Park - Salvage	7	*Mecinus janthinus*	Front Range Field Collection Site, CO	Uneclosed Adults	4 per replicate
No Choice Test	*Penstemon virgatus*	2	Rocky Mountain National Park - Salvage	2	*Mecinus janthinus*	NE Washington Collection Site via USDA	Eclosed Adults	4 per replicate
No Choice Test	*Penstemon secundiflorus*	1	Rocky Mountain National Park - Salvage	7	*Mecinus janthinus*	Front Range Field Collection Site, CO	Uneclosed Adults	4 per replicate
No Choice Test	*Penstemon secundiflorus*	2	Rocky Mountain National Park - Salvage	7	*Mecinus janthinus*	NE Washington Collection Site via USDA	Eclosed Adults	4 per replicate
No Choice Test	*Penstemon virens*	1	Rocky Mountain National Park - Salvage	7	*Mecinus janthinus*	Front Range Collection Site, CO	Uneclosed Adults	4 per replicate
No Choice Test	*Penstemon virens*	2	Rocky Mountain National Park - Salvage	7	*Mecinus janthinus*	NE Washington Collection Site via USDA	Eclosed Adults	4 per replicate
No Choice Test	*Mimulus guttatus*	1	Western Native Seed	7	*Mecinus janthinus*	Front Range Field Coll. Site, CO	Uneclosed Adults	4 per replicate

Experiment	Plant	Block	Source	#	Insect	Source	Stage	#
No Choice Test	*Mimulus guttatus*	2	Western Native Seed	7	*Mecinus janthinus*	NE Washington Collection Site via USDA	Eclosed Adults	4 per replicate
No Choice Test	*Nuttalanthus texana*	2	Boulder Co. Open Space - Seed	12	*Mecinus janthinus*	NE Washington Collection Site via USDA	Eclosed Adults	4 per replicate
Choice Test	*Linaria dalmatica*	1	University of Colorado (east campus) - Seed	3	*Mecinus janthinus*	Front Range Field Collection Site, CO	Uneclosed Adults	6 per replicate
Choice Test	*Penstemon virgatus*	1	Rocky Mountain National Park - Salvage	3	Same	Same	Same	Same
Choice Test	*Mimulus lewisii*	1	Western Native Seed	3	Same	Same	Same	Same
Choice Test	*Castilleja spp.*	1	Jefferson Co. Seed	3	Same	Same	Same	Same
Choice Test	*Linaria dalmatica,*	2	University of Colorado (east campus) - Seed	9	*Mecinus janthinus*	NE Washington Collection Site via USDA	Eclosed Adults	6 per replicate
Choice Test	*Mimulus lewisii*	2	Western Native Seed	9	Same	Same	Same	Same
Choice Test	*Castilleja spp.*	2	Jefferson Co. Seed	5	Same	Same	Same	Same
Choice Test	*Penstemon virgatus*	2	Rocky Mountain National Park - Salvage	9	Same	Same	Same	Same
Choice Test - *L. dalmatica L. vulgaris*	*Linaria dalmatica*	2	University of Colorado (east campus) - Seed	8	*Mecinus janthinus*	NE Washington Collection Site via USDA	Eclosed Adults	6 per replicate
Choice Test - *L. dalmatica/ L. vulgaris*	*Linaria vulgaris*	2	Rocky Mountain National Park - Salvage	8	Same	Same	Same	Same
Larval Transfer	*Linaria dalmatica*	2	University of Colorado (east campus) - Seed	3	*Mecinus janthinus* - larvae	NE Washington Collection Site via USDA	Reared in GH, larvae emoved from plants	29 larvae transferred

Experiment	Plant	Block	Source	#	Insect	Source	Stage	#
Larval Transfer	*Penstemon virgatus*	2	Rocky Mountain National Park - Salvage	3	*Mecinus janthinus* - larvae	NE Washington Collection Site via USDA	Reared in GH, larvae removed from plants	6 larvae transferred
Larval Transfer	*Mimulus lewisii*	2	Western Native Seed	3	*Mecinus janthinus* - larvae	NE Washington Collection Site via USDA	Reared in GH, larvae removed from plants	18 larvae transferred
Larval Transfer	*Scrophularia macrantha*	2	Harlequin Gardens Nursery	1	*Mecinus janthinus* - larvae	NE Washington Collection Site via USDA	Reared in GH, larvae removed from plants	2 larvae transferred
Larval Transfer	*Veronica spicata*	2	Harlequin Gardens Nursery	1	*Mecinus janthinus* - larvae	NE Washington Collection Site via USDA	Reared in GH, larvae removed from plants	2 larvae transferred
Larval Transfer	*Verbascum chiaxii* “Album”	2	Harlequin Gardens Nursery	1	*Mecinus janthinus* - larvae	NE Washington Collection Site via USDA	Reared in GH, larvae removed from plants	3 larvae transferred

APPENDIX C. Vouchers deposited in University of Colorado Herbarium (Specimens collected by N. Breiter)

Species	Family	Accession #	Co., State	Date Coll.	UTM Coord.	Elev.	Site Description
Penstemon secundiflorus	SCR	505746	Boulder, CO	9/16/04	13 T 0481508 UTM 4455316	5709 ft	Rabbit Mountain Open Space Park, NE of Lyons on N 53rd Street. Open grassland on west-facing hillside with *Yucca glauca* and *Andropogon gerardii*; elevation
Penstemon secundiflorus	SCR	505745	Jefferson, CO	8/08/04	13 S 0482509 UTM 4393659	6336 ft.	Mathew-Winters Open Space Park, S of Golden on Hwy. 93. Shrub-grassland on east-facing slope with *Cercocarpus montanus*, *Yucca glauca* and *Chrysothamnus* sp
Penstemon secundiflorus	SCR	505744	Boulder, CO	5/27/04	N 40.12104 W105.32471	6398 ft.	Private property, off Left Hand Canyon Rd and W of Heil Ranch Open Space. Area burned by Overland Wildfire in 2003, steep west-facing slope with granite outcrops with *Anisantha tectorum, Rosa woodsii* and *Artemisia frigida*
Penstemon virens	SCR	505743	Boulder, CO	5/27/04	N 40.12320 W 105.32145	6250 ft.	Sanabria private property, off Left Hand Canyon Rd and W of Heil Ranch Open Space. Area burned by Overland Wildfire in 2003, shaded bottom portion of slope with *Pinus ponderosa* and *Mahonia repens*
Linaria dalmatica	SCR	505742	Boulder, CO	5/27/04	N 40.12374 W 105.32101	6286 ft.	Sanabria private property, off Left Hand Canyon Rd and W of Heil Ranch Open Space. Area burned by Overland Wildfire in 2003, open west-facing slope with *Anisantha tectorum* and *Euphorbia myrsinites*
Linaria genistifolia	SCR	505741	Boundary, ID	9/09/04	11 U 0541735 UTM 5374687	2176 ft	Private property N of Sandpoint. Growing in dense stand of *Centaurea maculosa*
Penstemon procerus	SCR	505740	Ferry, WA	9/08/04	11U 0414686 UTM 5405999	1410 ft	Barstow railroad right of way on W side of Hodgson-Lakin Rd. N of Kettle Falls. Flat area with *Pinus ponderosa, Centaurea diffusa* and *Hypericum perforatum*
Penstemon procerus	SCR	505739	Pend Orielle, WA	9/09/04	11 U 0470329 UTM 5324183	2147 ft	Private property SW of Newport. Growing on edge of field of *Linaria dalmatica* and *Centaurea diffusa* with *Pinus ponderosa*

APPENDIX D. Site Observation Information

Site #	Date	Site Name	County, State	GPS Coordinates	Elev.	Site Characteristics	Target Plant	M.j. release date	Number of M.j. released	Source of M.j.
1	8/17/04	Mathew/Winters Open Space #1 (Near Golden, CO)	Jefferson, CO	13 S 482378 UTM 393715	6350 ft	Foothills, Shrub and grassland, east-facing slope	*L. dalmatica*	5/22/01 and 6/20/02	100 / 400	BC Canada via CO Insectory
2	8/17/04	Mathew/Winters Open Space #2 (Near Golden, CO)	Jefferson, CO	13 S 0482509 UTM 4393659	6336 ft	Foothills, Shrub and grassland, east-facing slope	*L. dalmatica*	Same as above.	Same as above.	BC Canada via CO Insectory
3	8/3/04	MJ-02-14, Rio Blanco Ranger District, White River National Forest (near Meeker, CO)	Rio Blanco, CO	13 N 275088 UTM 4424046	8359 ft	Aspen grove	*L. vulgaris*	6/18/02	200	CO Insectory
4	8/3/04	Main Marvine Crk Drainage, Rio Blanco Ranger District, White River NF (Near Meeker, CO)	Rio Blanco, CO	13 N 293098 UTM 4431339	8336 ft	Aspens and high native plant density and diversity	*L. vulgaris*	2004	100	Integrated Weed Control (private), MT
5	8/4/04	West Marvine Crk Drainage, Rio Blanco Ranger District, White River NF #1 (near Meeker, CO)	Rio Blanco, CO	13 T 0292514 UTM 4430456	8434 ft	Aspen, Pine, high meadow	*L. vulgaris*	6/26/02	200	BC Canada via CO Insectory
6	8/4/04	West Marvine Crk Drainage, Rio Blanco Ranger District, White River NF #2 (Near Meeker, CO)	Rio Blanco, CO	13 T 0292464 UTM 443076	8379 ft	West-facing slope, aspen	*L. vulgaris*	2004	100	Integrated Weed Control (private), MT

Site #	Date	Site Name	County, State	GPS Coordinates	Elev.	Site Characteristics	Target Plant	M.j. release date	Number of M.j. released	Source of M.j.
7	7/13/04	Lory State Park (Near Ft. Collins, CO)	Larimer, CO	13 N 483662 UTM 4493171	5800 ft	Foothills west of Horsetooth Reservoir on East facing slope in drainage	*L. dalmatica*	6/26/02	200	BC Canada via CO Insectory
8	9/4/04	Rabbit Mountain, Boulder Co. Open Space #1 (Near Lyons, CO)	Boulder, CO	13 T 0481451 UTM 4455384	5691 ft	Tall grass and Ponderosa pine habitat. High native plant diversity.	*L. dalmatica*	6/3/2003	80	Integrated Weed Control (private), MT
9	9/4/04	Rabbit Mountain, Boulder Co. Open Space #2 (near Lyons, CO)	Boulder, CO	13 T 0481508 UTM 4455316	5709 ft	Tall grass and Ponderosa pine habitat. High native plant diversity.	*L. dalmatica*	6/3/2003	80	Integrated Weed Control (private), MT
10	9/7/04	Cook Mountain, Colville National Forest #1 (near Republic, WA)	Ferry, WA	11 U 0383764 UTM 5394276	3982 ft	Open Ponderosa Pine with grassy understory, SW facing steep hillside	*L. dalmatica*	6/6/01	200	Collection and redistrib.
11	9/7/04	Cook Mountain, Colville National Forest #2 (near Republic, WA)	Ferry, WA	11 U 0383822 UTM 5393538	3666 ft	Open hillslope, West facing	*L. dalmatica*	5/29/02	200	Collection and redistrib.
12	9/7/04	Torboy Railroad Crossing, West Curlew Lake Road, (near Curlew Lake, WA)	Ferry, WA	11 U 0377111 UTM 5393143	2400 ft	Railroad right of way, West facing open hillslope	*L. dalmatica*	6/28/01	200	Collection and redistrib.

Site #	Date	Site Name	County, State	GPS Coordinates	Elev.	Site Characteristics	Target Plant	M.j. release date	Number of M.j. released	Source of M.j.
13	9/7/04	Danville Railroad Site (Danville, WA)	Ferry, WA	11 U 0390814 UTM 5428457	1737 ft	Railroad right of way, disturbed site	*L. dalmatica, L. vulgaris, L. genistifolia*	Natural dispersal from BC/ unknown date	Unknown	BC Canada
14	9/7/04	Buckboard Saloon Site (Danville, WA)	Ferry, WA	11 U 0389900 UTM 5428215	1756 ft	Road shoulder and south facing hillslope. Open Ponderosa pine overstory.	*L. dalmatica*	Natural dispersal from British Columbia/ unknown date	Unknown	BC Canada
15	9/7/04	Beck Site, Private Property (near Danville, WA)	Ferry, WA	11 U 0388060 UTM 5420281	2114 ft	Ponderosa pine and open grassy understory. South facing hillslope	*L. dalmatica*	Natural dispersal from British Columbia/ unknown date	Unknown	BC Canada
16	9/7/04	Danville Cemetary (near Danville, WA)	Ferry, WA	11 U 0389135 UTM 5427244	1817 ft	Open roadside strip	*L. dalmatica*	Natural dispersal from British Columbia/ unknown date	Unknown	BC Canada
17	9/7/04	Gracey Site (near Danville, WA)	Ferry, WA	11 U 0388811 UTM 54273337	1967 ft	Old hay field, abandonded last 4-5 years	*L. dalmatica*	Natural dispersal from BC/ unknown date	Unknown	BC Canada

Site #	Date	Site Name	County, State	GPS Coordinates	Elev.	Site Characteristics	Target Plant	M.j. release date	Number of M.j. released	Source of M.j.
18	9/8/04	Barstow Railroad Site (near Barstow and Kettle Falls, WA)	Ferry, WA	11 U 0414686 UTM 5405999	1410 ft	Ponderosa pine and open grassy understory.	*L. dalmatica*	5/29/02	200	Collection and redistrib.
19	9/8/04	Old Northport Airport (near Northport, WA)	Stevens, WA	11 U 0442755 UTM 5419505	1376 ft	Ponderosa pine and open grassy understory. Highly disturbed site.	*L. dalmatica,* *L. vulgaris*	Natural dispersal from British Columbia/ unknown date	Unknown	BC Canada
20	9/8/04	Abandoned orchard (between Northport and Kettle Falls, WA)	Stevens, WA	11 U 0432683 UTM 5409308	1369 ft	Ponderosa pine and open grassy understory.	*L. dalmatica*	Natural dispersal from British Columbia/ unknown date	Unknown	BC Canada
21	9/8/04	Railroad Right of Way (between Northport and Kettle Falls, WA)	Stevens, WA	11 U 0341560 UTM 5407242	1397 ft	Open railroad right of way surrounded by dense mixed conifer stand	*L. dalmatica*	Natural dispersal from British Columbia/ unknown date	Unknown	BC Canada
22	9/9/004	Roger's Site, Private Property (near Sandpoint, ID)	Boundary, ID	11 U 0541735 UTM 5374687	2176 ft	Open field dominated by knapweed. Surrounded by Ponderosa pine stand.	*L. genistifolia*	6/4/04	200	Collection and redistrib.

Site #	**Date**	**Site Name**	**County, State**	**GPS Coordinates**	**Elev.**	**Site Characteristics**	**Target Plant**	**M.j. release date**	**Number of M.j. released**	**Source of M.j.**
23	9/9/04	Newport, WA	Pend Orielle, WA	11 U 0494666 UTM 5335618	2168 ft	Ponderosa pine and open grassy understory.	*L. dalmatica*	2003 and 2004	400 total over 2 years	Collection and redistrib.
24	9/9/04	Private Field (near Newport, WA)	Pend Orielle, WA	11 U 0470329 UTM 5324183	2147 ft	Knapweed and toadflax dominated field surrounded by open stand of Ponderosa pine	*L. dalmatica*	2003 and 2004	Unknown	Collection and redistrib.

APPENDIX E. Greenhouse and field experiments data

No Choice Tests Data (greenhouse)

Species	Data Reported	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10	Rep 11	Rep 12	Rep 13	Rep 14
Linaria dalmatica	Feeding Score (max)	3	3	3	3	3	3	3	0	3	3	2	3	3	3
	Oviposition. Scars	21	57	40	232	25	157	113	N/A	134	35	139	56	125	72
	Larvae	12	2	7	3	4	19	3	N/A	55	6	48	22	45	42
Linaria vulgaris	Feeding Score (max)	3	1	3	3	3	3	3	3	3	2	3	3	2	1
	Oviposition. Scars	60	0	30	91	18	31	25	54	14	0	0	29	0	2
	Larvae	7	0	5	9	2	0	0	6	0	0	0	4	0	0
Mimulus guttatus	Feeding Score (max)	1	0	1	0	0	1	0	0	0	0	0	0	0	0
	Oviposition. Scars	0	0	1	2	0	0	0	0	0	0	0	0	0	0
	Larvae	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nuttalanthus texanus	Feeding Score (max)	2	2	1	2	1	1	1	1	1	1	2	2	N/A	N/A
	Oviposition. Scars	0	1	0	1	ND	0	0	0	0	6	0	0		
	Larvae	0	0	0	0	ND	0	0	0	0	0	0	0		
Penstemon secundiflorus	Feeding Score (max)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Oviposition. Scars	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	Larvae	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Penstemon virens	Feeding Score (max)	1	1	0	0	0	0	0	0	0	0	0	0	0	0
	Oviposition. Scars	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	Larvae	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Penstemon virgatus	Feeding Score (max)	1	1	0	1	0	0	0	0	0	N/A	N/A	N/A	N/A	N/A
	Oviposition. Scars	0	0	0	1	0	0	0	0	0					
	Larvae	0	0	0	0	0	0	0	0	0					

Choice Tests Data (greenhouse)

Species	**Data Reported**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**	**Rep 5**	**Rep 6**	**Rep 7**	**Rep 8**	**Rep 9**	**Rep 10**	**Rep 11**	**Rep 12**
Linaria dalmatica	Feeding Score (max)	3	3	3	3	3	2	3	3	3	3	3	3
	Oviposition. Scars	ND	ND	ND	154	ND	245	268	239	418	177	193	46
	Larvae	ND	ND	ND	84	ND	111	105	60	79	90	50	34
Mimulus lewesii	Feeding Score (max)	0	1	0	0	0	0	0	0	0	0	0	0
	Oviposition. Scars	ND	ND	ND	0	0	0	ND	0	0	0	ND	0
	Larvae	ND	ND	ND	0	0	0	ND	0	0	0	ND	0
Penstemon virgatus[1]	Feeding Score (max)	0	0	0	0	0	0	0	0	0	0	0	0
	Oviposition. Scars	ND	ND	ND	ND	ND	0	ND	ND	0	ND	ND	ND
	Larvae	ND	ND	ND	ND	ND	0	ND	ND	0	ND	ND	ND
Castilleja spp.	Feeding Score (max)	0	0	0	0	0	0	0	0	N/A	N/A	N/A	N/A
	Oviposition. Scars	ND	ND	ND	ND	ND	0	ND	ND				
	Larvae	ND	ND	ND	ND	ND	0	ND	ND				

ND = No data (In these tests, indicates that plant expired before end of 8-week observation period)
[1] *P. secundiflorus* substituted in replicate 12

Linaria dalmatica/Linaria vulgaris Choice Tests (greenhouse)

Species	**Data Reported**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**	**Rep 5**	**Rep 6**	**Rep 7**	**Rep 8**
Linaria dalmatica	Feeding Score (max)	3	3	3	3	1	3	3	2
	Oviposition. Scars	13	ND	0	6	0	ND	16	15
	Larvae	0	ND	0	0	0	ND	5	1
Linaria vulgaris	Feeding Score (max)	2	2	3	2	3	2	1	3
	Oviposition. Scars	25	35	5	0	30	32	2	28
	Larvae	0	12	1	0	7	0	1	0

Results of No Choice Tests (field experiments)

Species	Data Reported	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
Linaria dalmatica	Feeding Score (max)	2	1	1	0	1	1	1	2	1	1
	Oviposition. Scars	146	67	5	15	25	15	17	10	30	48
	Larvae	16	10	0	1	3	1	2	2	5	5
Penstemon secundiflorus	Feeding Score (max)	0	0	0	0	0	0	0	0	0	0
	Oviposition. Scars	0	0	0	0	0	0	0	0	0	0
	Larvae	0	0	0	0	0	0	0	0	0	0
Penstemon virens	Feeding Score (max)	0	0	0	0	0	0	0	0	0	0
	Oviposition. Scars	0	0	0	0	0	0	0	0	0	0
	Larvae	0	0	0	0	0	0	0	0	0	0

ND = No data

APPENDIX F. Site Observation Findings

Site #	Evidence of M.j. establishment	Native plants of interest at site	Survey done	Non-target herbivory?	Notes
1	Feeding marks on leaves, oviposition scars, larvae in stems	No native scrophs near site	N/A	No evidence found	Found evidence of weevil over large area, > 1 acre
2	Feeding marks on leaves, oviposition scars, larvae in stems	*Penstemon secundiflorus*	Inspected 40 stems of *P. secundiflorus.*	No evidence found	Penstemon co-occurred on slope with L. dalmatica and established weevils.
3	Could not find evidence of *M. janthinus* at site	*Castilleja* sp.	Inspected several stems in field.	No evidence found	
4	Could not find evidence of *M. janthinus* at site	*Castilleja* sp.	Inspected several stems in field.	No evidence found	Very high densities of accidental introduction *Brachypterolus pulicarius*
5	Feeding marks on leaves, oviposition scars, larvae in stems at close to exact release location	No native scrophs near site		No evidence found	Little evidence of weevil dispersal over larger area
6	Could not find evidence of *M. janthinus* at site	*Castilleja* sp.	Inspected several stems in field.	No evidence found	
7	Could not find evidence of *M. janthinus* at site	No native scrophs near site	N/A	N/A	
8	Feeding marks on leaves, oviposition scars, adults in stems	No native scrophs near site	N/A	N/A	
9	Feeding marks on leaves, oviposition scars, adults in stems	*Penstemon secundiflorus*	Inspected several stems in field.	No evidence found	
10	Feeding marks on leaves, oviposition scars, larvae and adults in stems	No native scrophs near site	N/A	N/A	High density and large dispersal area of M.j.
11	Feeding marks on leaves, oviposition scars, larvae and adults in stems	No native scrophs near site	N/A	N/A	Moderate density and medium dispersal of M.j., Also found *Calophasia lunula* at site.

Site #	Evidence of M.j. establishment	Native plants of interest at site	Survey done	Non-target herbivory?	Notes
12	Feeding marks on leaves, oviposition scars, larvae and adults in stems	No native scrophs near site	N/A	N/A	Very disturbed site.
13	Feeding marks on leaves, oviposition scars, larvae and adults in stems on all species	No native scrophs near site	N/A	N/A	M.j. collection site. Very little toadflax remaining. High densities of M.j.
14	Feeding marks on leaves, oviposition scars, larvae and adults in stems	No native scrophs near site	N/A	N/A	M.j. collection site. Very little toadflax remaining. High densities of M.j.
15	Feeding marks on leaves, oviposition scars, larvae and adults in stems	No native scrophs near site	N/A	N/A	Collection site.
16	Feeding marks on leaves, oviposition scars, larvae and adults in stems	No native scrophs near site	N/A	N/A	Very few natives, disturbed site.
17	Feeding marks on leaves, oviposition scars, larvae and adults in stems	No native scrophs near site	N/A	N/A	Very few natives.
18	Feeding marks on leaves, oviposition scars, larvae and adults in stems	*Penstemon procerus* (found patches both within infestations and outside of infestations)	Examined leaves, collected and dissected *P. procerus* stems (17 stems from site w/in weevil population on *L. dalmatica patch* and 26 stems from site outside of *L. dalmatica* patch	No evidence found	Collection site. Very high densities of M.j.
19	Feeding marks on leaves, oviposition scars, larvae and adults in stems	*Penstemon procerus*	Examined leaves, collected and dissected 15 *P. procerus* stems from w/in *L. dalmatica* patch	No evidence found	Was first US site where M.j. found naturally dispersed from Canada in 1999. Collection site since 2000.
20	Feeding marks on leaves, oviposition scars, larvae and adults in stems	*Penstemon procerus* (found patch 10-15 meters from toadflax infestation)	Examined leaves, collected and dissected 15 *P. procerus* stems from w/in *L. dalmatica* patch	No evidence found	Very high M.j. density on small patch of toadflax.

Site #	Evidence of M.j. establishment	Native plants of interest at site	Survey done	Non-target herbivory?	Notes
21	Feeding marks on leaves, oviposition scars, larvae and adults in stems	*Penstemon procerus* (found patch 15-20 meters from toadflax infestation)	Examined leaves, collected and dissected 9 *P. procerus* stems from w/in *L. dalmatica* patch	No evidence found	Many natives in forest stand.
22	Feeding marks on leaves, oviposition scars, larvae and adults in stems	No native scrophs near site	N/A	N/A	
23	Feeding marks on leaves, oviposition scars, larvae and adults in stems	No native scrophs near site	N/A	N/A	Moderate establishment of M.j. Significant native plant component in surroundings.
24	Feeding marks on leaves, oviposition scars, larvae and adults in stems	*Penstemon procerus* (found patch 15-20 meters from toadflax infestation)	Examined leaves, collected and dissected 14 *P. procerus* stems from w/in *L. dalmatica* patch	No evidence found	Very dense, large patch of toadflax with moderate - low establishment of M.j.