POST-RELEASE ASSESSMENT OF CLASSICAL BIOLOGICAL CONTROL OF CANADA THISTLE (*CIRSIUM ARVENSE*) IN THE WESTERN UNITED STATES

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ABSTRACT

Classical biological control of weeds has been used almost 50 years to control *Cirsium arvense* (Canada thistle) in the United States. However, few field studies have assessed the efficacy of approved biological control agents, a stem-galling fly *Urophora cardui* and stem-mining weevil *Hadroplontus litura*. We reviewed literature on *C. arvense* control, and between 2008 and 2012, monitored release transects of *U. cardui*, *H. litura*, both species, or no insects released at *C. arvense* infestations across the western United States. At each study transect (n = 87), we measured *U. cardui* parasitism pressure, biological control agent establishment and abundance, vegetation cover, *C. arvense* density, and abiotic variables; each as potential factors in inter-annual *C. arvense* density fluctuation. We found that *U. cardui* galls are attacked by five parasitoid species at low rates and that *C. arvense* infestations are negatively affected by perennial grass competition and not by biological control agent herbivory.

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Abstract

Cirsium arvense (Canada thistle) is considered one of the most significant invasive plant species worldwide and *C. arvense* control efforts have been conducted since nearly a century. This literature review contains a brief introduction to *C. arvense* biology and invasiveness, including species description, habitat conditions, and plant reproduction/spread. The second section contains discussion of common *C. arvense* management strategies (e.g., chemical, mechanical, and cultural) and the limitations of each. The third section contains the history of classical biological weed control used, since almost 50 years, to control *C. arvense* in the United States. We focus our discussion on the two approved biological control agents, the stem-galling fly *Urophora cardui* and the stem-mining weevil *Hadroplontus litura* on *C. arvense*. In the final section, we summarize the current status of *C. arvense* control programs and suggest areas of future research.

Introduction

The herbaceous invasive plant, *Cirsium arvense* (L.) Scop. (Canada thistle, California thistle, Creeping thistle, hereafter CT) is a perennial plant in the Asteraceae family and is native to south-eastern Europe, western Asia, and northern Africa (Donald, 1990; Moore, 1975; Morishita et al., 1999; Skinner et al., 2009). Genetic and historical data indicate that CT was introduced to North America with the first European settlers in the 1600s from Western Europe as contaminant of grain seed (Jacobs et al., 2006). *Cirsium arvense* was likely again introduced from Eastern Europe into the prairie states (Guggisberg et al., 2012).

Cirsium arvense grows in temperate environments where soils are disturbed (e.g., eroded, previously flooded, or heavily grazed) but preforms poorly in undamaged pastures (Bossard et al., 2000; Evans, 1984; Zouhar, 2001). The plant prefers open meadows and riparian areas with access to moisture (Zouhar, 2001). Seedlings of CT produce basal rosettes that bolt in late spring and expire each fall (Gange et al., 2012). *Cirsium arvense* is a clonal and dioecious plant that grows 30 to 220 cm tall. *Cirsium arvense* grows spiny leaves that alternate on an elevated and freely branched stem which can produce up to 1500 seeds (Hay, 1937). Seed dispersal can be up to one kilometer from the parent plant, or further, if carried away in flowing water (Bostock and Benton, 1979; Nuzzo, 1997). Seeds can spread via deposition in animal feces (esp. waterfowl), in soil attached to automobiles or farm equipment, and as contaminant in crop seed, hay, or packing material (Hayden, 1934; Nuzzo, 1997). Though, seeds are considered the primary source for new invasions (Bodo Slotta et al., 2010), CT can also spread through horizontal subterranean shoots that generate numerous flowering ramets (Bourdot et al., 1995). These clonal ramets, along with secondary stem bud activation, allow herbaceous perennial weeds to escape chemical, cultural, mechanical, and biological methods (Anderson et al., 2010). Although CT uses clonal reproduction, due to CT efficient outcrossing breeding system and ability to interbreed with previously isolated populations, the species retains a high level of genetic diversity in North America (Guggisberg et al., 2012).

Cirsium arvense has been an invasive species around the world for more than a century (Holm et al., 1977). It is considered one of the "world's worst weeds" and is distributed throughout temperate regions of the globe including: Europe, Africa, western and central Asia, India, Japan, China, North and South America, New Zealand, Tasmania, and

Australia (Friedli and Bacher, 2001; Holm et al., 1977; Hulten, 1968; Moore, 1975; Nuzzo, 1997; Pritekel et al., 2006; Tiley, 2010). *Cirsium arvense* was listed as a noxious weed species beginning in the 1970s (Nuzzo, 1997) and maintains noxious weed status in at least 41 U.S. states; it is not currently officially distributed in some south-eastern states and Hawaii (USDA, 2013).

Cirsium arvense is especially invasive in agro-ecosystems and natural areas with severe economic losses to crop yields, rangeland, and pasture productivity (Hartley and James, 1979; McLennan et al., 1991; Mitchell and Abernethy, 1993; Moyer et al., 1991; O'Sullivan et al., 1982). The cause of economic loss can range from the cost of herbicides to the vaccination of grazing animals wounded by thistle spines (Gourlay, 2004). Up to 60% yield loss have been reported in CT infested crops and the global annual monetary losses due to CT infestation have been estimated at 320 million US$ (Bailey et al., 2000; Müller and Nentwig, 2011). *Cirsium arvense* success as a weedy invasive in North America (Pritekel et al., 2006) may in-part be due to release from specialist natural enemies found in the native Eurasian range (Keane and Crawley, 2002).

Management of *C. arvense*

Attempts to control CT date back more than one hundred years (Burrhill, 1890); however, management of CT has thus far been generally unsuccessful (Cripps et al., 2011). Perennial plants, such as CT, provision nutrients in roots that allow regrowth of above ground biomass in subsequent years. In order to eradicate the plant or enhance restoration outcomes, it is imperative to exhaust root supplies. This involves multiple years and various practices to pressure the plant into controlled development or elimination. Although *C. arvense* in the

North American invaded range has evolved greater resistance to abiotic stressors (e.g., light reduction) than the European ancestor populations (Guggisberg et al., 2013), grasses, fast-growing annual forbs, or functionally similar native forbs can be used as cultural control to negatively affect CT growth and reproductive capacity (Burns et al., 2013). Planting several native species that compete with thistle plants for available light and nutrients can result in reduced seedling establishment from the seed bank but is most effective only in newly emergent habitat (Norland et al., 2013).

Chemical control can be used to control CT and several herbicides are accessible to facilitate management. However, herbicide application can be difficult in remote, rugged, or aquatic environments (Krueger-Mangold et al., 2002) and retreatment is often required due to many herbicides particular application timing, individual plants overlooked during application, and herbicides mainly affect above-ground growth leaving root stores undamaged (Müller and Nentwig, 2011). Economic and environmental constraints on chemical management of CT often result in increased interest in other control methods (Burns et al., 2013).

Mechanical control involving tilling, have a propensity to yield further plant development and spread. *Cirsium arvense* taproot can grow nearly one meter deep, produce a new shoot every 5cm, and even a 6 mm root segment has energy to regenerate into a new plant. These plant characteristics cause mechanical tillage to be an unproductive management method. In fact, cultivation or tillage stimulates the growth of horizontal roots and the quantity of standing shoots borne by the horizontal runners increase; thus, the plant is locally spread (Hayden, 1934). However, mechanical control via mowing can be an effective tool if completed multiple times, and multiple years, with the intention of stressing the plant into

depleting root stores meant for regrowth. Mowing in the fall during seed dispersal can scatter seeds farther, and ought to be avoided. Also, a single mowing event results in a compensatory response via increased root mass (Mitchell and Davis, 1996). Mowing can reduce competition from neighboring plant species and positively affect CT growth (Edwards et al., 2000). However, herbicide application following mowing is a valuable combination for additional plant stress (Beck, 2008).

Cirsium arvense is considered a difficult target for classical biological control of weeds due to a number of traits, including the fact that it is also a major weed in its native range (Schroeder et al., 1993). Nonetheless, classical biological control of CT has been attempted in the United States since 1966 (Julien and Griffiths, 1998), probably because the benefits of a potentially successful biocontrol program against the economically significant plant would offset the relatively high probability of failure (Page and Lacey, 2006; Paynter et al., 2012). Biological control can be efficiently implemented to repress CT infestation, but it is most successful when combined with other treatments. Numerous agents have been introduced for the biological control of CT, whether deliberate or accidental. Our study focuses on two biological control insect species known to have questionable impact on the target weed.

A classical biological control program intended to manage *C. arvense* in North America was initiated in 1959, principally by Canadian researchers (Schroder, 1980). The bulk of phytophagous insects considered for release on CT as biological control agents were discovered in surveys within Western Europe from 1961 to 1964 (Zwölfer, 1965). Invertebrate biological control agents have been released across North America with modest success (McClay et al., 2002). These biological control agents are gall-formers and stem-

miners intended to diminish plant growth, competitive ability, reproductive seed production, and plant density (Zwölfer, 1985).

The initial biological control agent released in North America was *Altica carduorum.* It is a flea beetle introduced into Canada 1963, Great Britain in 1969, New Zealand in 1979, and the United States in 1966 (Julien and Griffiths, 1998). This insect may have failed to establish in North America due to predation pressure on the beetle, and poor climate matching of the native and introduced ranges (Peschken, 1977; Peschken et al., 1970).

The second intentional biological control agent released was the stem-mining weevil *Hadroplontus litura* (F.) (Coleoptera, Curculionidae) from its native range (Germany, France, Italy, and Switzerland) into Canada in 1965, New Zealand in 1976, and the U.S. in 1971 (Julien and Griffiths, 1998; Zwölfer and Harris, 1966). *Hadroplontus litura* is a univoltine weevil that overwinters in soil litter and emerges in spring, to feed on CT rosette foliage. Females lay eggs in the midrib of leaves and hatching larvae mine into stems and consume parenchymatic pith (Peschken and Wilkinson, 1981). Mature larvae mine toward the root crown and exit the stems during summer to pupate in the soil (Rees, 1990).

Rhinocyllus conicus (Froel.) (Coleoptera: Curculionidae) was released into Canada in 1968 (Julien and Griffiths, 1998). The oligophagous capitulum herbivory of *R. conicus* reduces the number of seeds per stem. However, because established CT populations use vegetative reproduction, seed reduction has minor effects compared to vegetative control methods (Leathwick and Bourdot, 2012). An additional limitation of *R. conicus* as a biological control agent is its broad host selection of thistle species, known to produce non-target effects (Louda et al., 1997). Also, the specific effect on CT is not known (Cripps et al., 2011).

Larinus planus (F.), a bud weevil, was screened in Europe as a potential biological control agent. It was accidentally introduced to the United States in the 1960's (first recorded present in 1971) and after further evaluation (McClay, 1990), *L. planus* was widely redistributed (White, 1972). However, the weevil has since shown significant non-target effects on native North American thistle species (Louda and O'Brien, 2002).

Urophora cardui (L.), a stem-gall fly in the family Tephritidae, was introduced from France, Austria, Finland, and Germany (McClay et al., 2002) for the biological control of CT in North America via releases in Canada in 1974 and the United States in 1977 (Harris and Shorthouse, 1996; Peschken and Harris, 1975). It is native to Central Europe where it now has a Palearctic distribution (Seitz and Komma, 1984). *Urophora cardui* is highly host specific and has no reported non-target effects in North America (Zwölfer and Harris, 1971). It is most abundant in moist shaded areas with scattered CT plants (Winston, 2005). *Urophora cardui* adult females lay eggs on leaves and shoots. Endophytophagous larvae tunnel into the stem and form a gall (Lalonde and Shorthouse, 1985). The gall larvae are not well adapted to survival in the presence of other thistle control methods (Daniels, 2004).

Lema cyanella (L.) (Coleoptera: Chrysomelidae) is a leaf-feeding beetle released into Canada in 1978, and New Zealand in 1983 (Julien and Griffiths, 1998). During host specificity testing, *L. cyanella* showed some non-target host feeding, but in negligible amounts. It feeds on CT both as a larvae and adult and has high reproductive rates with a large distribution in the native range (Peschken and Johnson, 1979). However, this leaf-feeding beetle is not in the United States, and failed to establish in Canada (Cripps et al., 2011).

A thistle defoliating beetle, *Cassida rubiginosa* (Müller) (Coleoptera: Chrysomelidae), is distributed in southern Canada to the U.S. states of Virginia and westward to Michigan.

Overwintering adults initially become active in late winter, and oviposit in early spring. The next generation of adults appear later that spring, and can be seen on thistles foliage as late as November (Ward and Pienkowski, 1978). In the field, adults may oviposit on CT regardless of host stem abundance and densities of *C. rubiginosa* vary within, and among, CT patches. *Eucelatoria dimmocki* (Aldrich) (Diptera: Tachinidae), *Spilochalcis side* (Walker) (Hymenoptera: Chalcididae), and *Itoplectis conquisitor* (Say) (Hymenoptera: Ichneumonidae) are known to parasitize *C. rubiginosa* larvae (Tipping, 1993). Despite this parasitism pressure, *C. rubiginosa* can significantly reduce biomass and survival of CT host plants. Long-term control of CT is achievable with *C. rubiginosa* (Ang et al., 1995) and the beetle a remains promising biological control agent across the globe (Asadi et al., 2013). However, complimentary methods of CT control are still required with releases of *C. rubignosa* to prevent substantial yield loss in crops (Asadi et al., 2013).

In recent surveys of arthropods feeding on *Cirsium arvense*, five of the eight species discovered are root-feeders (Gourlay, 2004). Members of this feeding guild (i.e., mining insects), have been shown to reduce the number of seed-heads produced and interrupt the clonal growth of true thistles. Thus, mining can negatively affect the net reproductive output of a plant or colony (Peschken and Derby, 1992). Although plant competition unaided often has a greater harmful effect on true thistles than these specialized endophytophagous herbivores, as with the study on the root boring weevil *Apion onopordi*, weevils can reduce the total above-ground biomass over time, and aid in plant competitors' ability to diminish host plant root growth. This reduced biomass can translate to moderated thistle performance in the form of lessened plant vigor and competitive capability (Friedli and Bacher, 2001).

Today, the stem-mining weevil *H. litura*, is one of the only biological control agents approved for release in the United States (Winston, 2005).

When *H. litura* is combined with other stressors (e.g., herbicides or competition) in greenhouse conditions, weevil herbivory may cause significant root biomass reduction (Collier et al., 2007; Ferrero-Serrano et al., 2008; Sciegienka et al., 2011). During garden studies, a single *H. litura* larva was not enough to reduce plant vigor; however, with higher larval attack levels, host plants were considerably smaller. Larval attack of thistle grown in greenhouse conditions under medium stress was associated with loss of thistle vitality. In a laboratory setting of low light and humidity, healthy CT lived approximately one month longer than those mined (Zwölfer and Harris, 1966).

Hadroplontus litura weevils establish well at release sites in North America and limit overwinter survival of CT if plant vigor is reduced by unfavorable growing conditions and larval attack by *H. litura* impairs or kills a thistle stem (Zwölfer and Harris, 1966); however, these weevils have a low dispersal rate and reproductive ability that limit destructive capacity and cause weevil herbivory to be generally ineffective (Peschken and Beecher, 1973) and cause no detectable effects on CT populations (Zwölfer and Harris, 1966). While larval mining produce holes in CT stems that facilitate secondary damage by pathogens, these pathogens suitability as biological control agents remain in question (Müller et al., 2011) and studies have shown that *H. litura* has little impact on CT in the field (Peschken and Wilkinson, 1981; Reed et al., 2006; Zwölfer and Harris, 1966), likely due to *H. litura* larvae feeding on nonessential plant tissues in the midpoint of the stalk (e.g., pith) (Peschken and Wilkinson, 1981). At high attack rates of up to 12 larvae per stem (3 to 6 larvae is typical), *H. litura* larvae combine individual mines and stem swelling or formation of an indistinctive gall

at the stem base is observed. Larvae can then be localized by the gall, after which the host plant continues to grow normally (Zwölfer and Harris, 1966). *Cirsium arvense* can survive attack by *H. litura* and can even kill *H. litura* larvae through enclosure in callus tissue.

Although some previous field testing of *H. litura* on CT has been linked with plant number declines (Montana) (Peschken and Derby, 1992; Rees, 1990), others have found no detrimental effects due to a lack of change in *C. arvense* shoot development among locations with high and low concentrations of *H. litura* (Reed et al., 2006). In fact, plants with higher levels of *H. litura* mining have greater main stem biomass, number of side shoots, and seed-heads than plants with lighter levels of attack if the soil nutrient availability is adequate; thus, *H. litura* has been deemed a 'relatively weak' biological control agent (Burns et al., 2013).

Aside from *H. litura*, the only other biological control agent currently approved for release in the United States is *U. cardui* (ISDA, 2014; Winston, 2005). Galls formation initiated by *U. cardui* larvae causes galls to act as a metabolic sink of plant resources (Harris and Shorthouse, 1996) that would otherwise been allocated to improving plant growth and reproduction (Thibodeau, 1985). Gall formation can result in reduced fitness and competitive ability of the host plant (Harris and Shorthouse, 1996). During pre-release assessments of *U. cardui,* roots of single and double-galled plants weighed 65% and 78% less respectively, than roots of unattacked plants. The combined above-ground straw weight (i.e., stem and foliage weight) was 47% and 58% respectively, less than that of stems without galls (Peschken and Harris, 1975). Galls absorb needed plant nutrients, resulting in stunted growth, but minimal impact overall (Harris and Shorthouse, 1996). Larvae change nutrient composition of gall tissue and draw carbohydrates and nitrogen away from shoot growth and rhizome formation (Daniels, 2004).

Although *U. cardui* populations in higher latitudes have higher infestation rates due to narrow climactic windows causing better synchronization with the host plants (Frenzel et al., 2000), gall formation has been shown to be limited at high altitudes by low temperatures (Freese and Zwolfer, 1996). Also, despite *U. cardui* establishment in North America (McClay et al., 2002) with populations that can reach great local abundances, it does not have any measurable impact in the field (Fay et al., 1996; Forsyth and Watson, 1984; Harris and Shorthouse, 1996; Julien, 1987; Peschken and Derby, 1992).

Some limitation as a biological control agent is due to the constrained oviposition strategy of *U. cardui*. For example, a study conducted in Canada found that *U. cardui* occupied only 1% of available host stands after its introduction (Zwölfer, 1994). Effects of *U. cardui* on CT growth are assumed to be small because flies attack plants only after stem maturation is nearly 80% complete and larval herbivory time is not long enough to significantly damage stems (Forsyth and Watson, 1984; Forsyth, 1984; Harris and Shorthouse, 1996). Gall size affects the fitness of the gall forming larvae; larger galls result in greater larval fitness. The optimal clutch size hypothesis states that ovipositing females may lay more eggs in a single gall in highly populated thistle stands (Freese and Zwolfer, 1996). While this dynamic may be beneficial to gall-fly larvae, it results in reducing host stem utilization in dense gall-fly populations and reduced host plant metabolic use of small galls in sparse gall-fly populations. *Urophora cardui* is considered of little biological control value in most agricultural situations (Cripps et al., 2011; Harris and Shorthouse, 1996) due, in part, to some degree of host plant compensation via increased rates of photosynthesis (Fay et al., 1996) and a lack of local population stability with large variations in population abundances (Cripps et al., 2011).

Overwintering galls are exposed to destruction by deer, mice, and phytophagous insects. Adult *U. cardui* have a short lifespan of two to three weeks and have a limited probability of finding a new host plant patch within a heterogeneous geography thus, egg-laying female flies are mostly restricted to a single plant patch (Eber and Brandl, 1996). The mortality rate is high among adult flies within CT patches and during dispersal since tephritid flies compose 5% of total prey acquired by spiders found in thistle patches, with an estimated 10 predatory spiders per plant (Aluja and Norrbom, 2000; Rauh, 1994). All of these facts contribute to negligible *U. cardui* impact in the field (Peschken et al., 1982).

Summary and future work

After implementing biological control for more than four decades, there is little indication for successful control of the weed elsewhere (Fowler et al., 2000; McClay et al., 2002). The post-release impact of *U. cardui* (Peschken et al., 1982), *H. litura* (Hein and Wilson, 2004; Larson et al., 2005; Peschken and Wilkinson, 1981; Reed et al., 2006; Rees, 1990), and both species combined (Markin and Larson, 2013; Peschken and Derby, 1992) has been assessed previously in the U.S. and elsewhere. When combined, attack by *U. cardui* and *H. litura* reduce the number of buds and root dry weight of *C. arvense*, but there is no effect on the number of stems, seed-heads produced, or on the above-ground weight of CT and it has been determined that there is no significant difference in thistle populations when combining *U. cardui* with *H. litura* (Peschken and Derby, 1992). This requires further testing with a monitoring program to gain additional information in solidifying these findings.

Classical biological control of *C. arvense* is one of the oldest weed biological control programs in the United States. But even for the most common biological control agents, *U.*

cardui and *H. litura*, there are few studies assessing their post-release impact on the target weed (Julien, 1987; Peschken and Derby, 1992). Previous studies have found that only 10% of gall density variation was explained by light, moisture, wind, alternative host plant density, and patch size (Frenzel et al., 2000). Also, resource accessibility and natural enemies are frequently mentioned mechanisms that affect competitive capacity of intrusive species; however, coinciding effects on host plant dynamics are seldom assessed in or based on field data. Parasitism is considered to be one of the key factors determining impact of *U. cardui* on its host plant (Abrahamson et al., 1983); however, the assessment of mortality factors for *U. cardui* has had little to no investigation. This mortality factor could be preventing the insect populations from healthy establishment, resulting in low reproductive rate and sluggish diffusion capability (Peschken and Beecher, 1973).

Despite mounting inquiries into the true effect of conventional biological control, the majority of studies fail to assess the synchronized influence of endogenous (e.g., stem density) and exogenous (e.g., precipitation) elements on fluctuations in target weed abundance in introduced ranges. Additional survey of insect release study sites is necessary to verify and improve data regarding the abundance and effectiveness of biological control on CT in the western United States via assessment of CT performance, population size, and vegetation community change after biological control agent release. Also, to account for environmental factors in herbivore species effectiveness, or lack thereof, investigation into community interaction is necessary and prompts further evaluation.

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CHAPTER 2: THE PARASITOID COMPLEX OF THE CANADA THISTLE BIOLOGICAL CONTROL AGENT *UROPHORA CARDUI* IN THE WESTERN UNITED STATES

Abstract

The European stem-galling fly, *Urophora cardui* (L.), has been released for the classical biological control of *Cirsium arvense* (Canada thistle) in the United States for since 1977. Previous studies on the impact of *U. cardui* on *C. arvense* in North America and New Zealand found that it varied and that is was generally limited. One of the factors that may limit the efficacy of *U. cardui* as a biological control agent may be parasitism in the areas where the fly has been introduced, but there are few studies that have assessed the parasitism pressure. Between 2010 and 2012, we sampled *U. cardui* galls from 35 field sites in the United States following a standardized collection protocol at *C. arvense* infestations in Washington State (n = 3), Oregon (n = 1), Idaho (n = 9), Montana (n = 6), North Dakota (n = 1), and South Dakota (n = 15). All galls were measured and kept individually throughout the winter months to rear out all emerging insects during the following spring. Insect specimens were identified to the lowest taxonomic level possible and separated into *U. cardui* gall inquilines and parasitoids. We assessed the parasitoid species composition and relative abundance of each species, and the number of emerging *U. cardui* from parasitized and healthy galls as a function of gall size. We found that *U. cardui* galls are attacked by at least five parasitoid species but only at low rates. By far the most abundant parasitoid of *U. cardui* found in our study was the European *Pteromalus elevatus* (Walker), which also attacks *U. cardui* in its native range. Overall, only 11.4% of all galls were attacked by parasitoids, less than half the proportion of galls found parasitized in the native range of *U. cardui*. We do not consider parasitism to be a factor that limits the control efficacy of *U. cardui* in the western United States.

Introduction

Elton (1958) classically defined biotic resistance as interactions of native species in a community with non-native species that slow the growth of those non-native species. According to that definition, natural enemies in the introduced range of a biological weed control agent could influence the control potential through predation or parasitism (Goeden and Louda, 1976; Paynter et al., 2010). Similarly, biotic resistance, or a lack thereof, is frequently mentioned as a mechanism that can affect the competitive ability of the non-native species (Uesugi and Kessler, 2013).

A recent review of biological weed control programs in New Zealand suggested that program success was associated with biotic resistance via parasitism (Paynter et al., 2010). In that review, one out of nine biological control agents categorized as 'effective' was parasitized, whereas eight of fifteen biological control agents categorized as 'unsuccessful' were parasitized. Paynter et al. (2010) concluded that biological control agent ability to suppress target weed populations is negatively associated with parasitism. Between the two mechanisms of biotic resistance that could affect biological control agents, parasitism is often believed to have stronger population-level effects than predation (Daniels, 2004; Szentesi and Jermy, 1990).

Low levels of host concealment (e.g., gall structures) are associated with an increased acquisition of parasitoids (Hill and Hulley, 1995). Studies on gall-forming weed biological control agents for *Chondrilla juncea* (L.) and *Euphorbia esula* (L.), for example, have shown that parasitism in the introduced range can render the respective biological control agents ineffective (Caresche and Wapshere, 1975; Horner, 2002; Lym and Carlson, 2009). Even though the gall midge, *Cystophora schmidti* (Rübsaamen), introduced for the biological

control of *C. juncea*, is considered very abundant and widespread, in its introduced range in the United States, heavy parasitism causes the gall midge to have no effect on the invasive plant (Littlefield and Barr, 1981; McCaffery, 1996).

Parasitism is generally considered one of the key factors determining the impact of gall flies on host plants (Abrahamson et al., 1983). For the European stem-galling fly *Urophora cardui* (L.) (Diptera, Tephritidae), host specific to *Cirsium arvense* (L.) Scop. (Canada thistle, California thistle, Creeping thistle, hereafter CT), it is known that populations in the native range noticeably fluctuate between years, in part due to high local extinction and colonization rates (Eber and Brandl, 1994; Eber and Brandl, 1996). Previous studies of *U. cardui* were initiated to clarify reasons for low levels of host plant impact and usage. These studies found that *U. cardui* is limited by a lack of shade and moisture, low temperatures at increasing altitudes, and a <50% establishment rate; yet these factors only explained 10% of observed gall density variation (Eber and Brandl, 1994; Freese and Zwölfer, 1996). Other studies found *U. cardui* limited by its own complex and rigid selection behavior (Goeden, 1987) or egg mortality (Freese, 1997). Outside of these abiotic and intraspecific limitation factors, parasitism rates up to 100% for local *U. cardui* populations are believed to partly drive the observed *U. cardui* population fluctuations in Europe (Eber and Brandl, 1997; Frenzel et al., 2000; Schlumprecht, 1990; Zwölfer, 1979; Zwölfer and Arnold-Rinehart, 1993). However, few studies have evaluated gall ineffectiveness in the introduced range due to interspecific mortality factors (e.g., parasitism).

Urophora cardui has been introduced as a classical biological control agent for CT in New Zealand and North America, and parasitism of the gall fly has been studied in the native European range (Basov, 2002; Eber and Brandl, 1994; Eber and Brandl, 1996; Frenzel et al.,

2000; Johannesen and Seitz, 2003b; Schlumprecht, 1989; Zwölfer, 1979; Zwölfer et al., 2007), in Canada (Peschken et al., 1997; Peschken et al., 1982), and in New Zealand (Paynter et al., 2010), but not in the United States, with the exception of Hoebeke and Wheeler Jr. (1996) who mention that parasitism may be a mortality factor for *U. cardui* in the United States.

Here we provide data on *U. cardui* parasitism rates and parasitoid complex composition on a large spatial scale for gall fly populations in the western United States. We were interested in the question of whether parasitism by native or exotic parasitoids could be a factor that limits the population growth or even leads to the local extinction of *U. cardui*, as is the case in its native range (Zwölfer et al., 2007). Our specific objectives were to identify the complex of parasitoid species that utilize *U. cardui* galls, their respective attack rates, and the consequences of parasitoid attack on fly survival.

Materials and methods

STUDY SYSYEM

Cirsium arvense is a perennial herbaceous plant in the Asteraceae family, native to south-eastern Europe and the eastern Mediterranean (Donald, 1990; Moore, 1975; Morishita et al., 1999; Skinner et al., 2009). It is considered one of the "world's worst weeds" and is distributed throughout temperate regions of the globe (Friedli and Bacher, 2001; Holm et al., 1977; Moore, 1975; Pritekel et al., 2006; Tiley, 2010). Seedlings of CT produce basal rosettes that bolt in late spring and die back in fall (Gange et al., 2012). *Cirsium arvense* is a clonal and dioecious plant that can produce several hundred ramets with stems up to 220 cm in height. Stems have spiny leaves that alternate, branch freely, and produce up to 1500 seeds

(Hay, 1937). Seed dispersal can be up to one kilometer from the parent plant or more, if carried by wind or flowing water (Bostock and Benton, 1979; Nuzzo, 1997). Plants also reproduce vegetatively through horizontal subterranean shoots that generate numerous flowering ramets (Bourdot et al., 1995).

Cirsium arvense grows in temperate environments where soils are disturbed (e.g., eroded, previously flooded, or heavily grazed) but performs poorly in undisturbed pastures (Bossard et al., 2000; Evans, 1984; Zouhar, 2001). The plant prefers meadows, riparian areas and habitats with access to moisture. Rangeland or crops invaded by *C. arvense* (Donald, 1990; Jacobs et al., 2006) result in economic losses (Hartley and James, 1979; McLennan et al., 1991; Mitchell and Abernethy, 1993; Moyer et al., 1991; O'Sullivan et al., 1982).

The gall-forming fruit fly, *U. cardui*, is native to central Europe (Seitz and Komma, 1984) and was first introduced for the biological control of CT into the U.S. in 1977 (Julien and Griffiths, 1998; Lalonde and Shorthouse, 1984; Peschken et al., 1997). *Urophora cardui* is host specific to *C. arvense* and, to date, there are no reports on non-target plant use (Zwölfer and Harris, 1971). The flies lay eggs in the upper parts of nearly mature stems that result in multi-chambered galls in which up to 14 fly larvae develop and pupate. Flies overwinter and emerge from galls the following spring.

Urophora cardui galls are considered a plant metabolic sink which may result in reduced CT fitness and competitive ability (Harris and Shorthouse, 1996; Thibodeau, 1985). Although *U. cardui* is well established in North America and locally abundant (McClay et al., 2002), there is no or little measureable impact in the field (Fay et al., 1996; Forsyth and Watson, 1984; Harris and Shorthouse, 1996; Julien, 1987; Peschken and Derby, 1992).

SITE SELECTION

Cirsium arvense infestations for *U. cardui* gall collection were chosen following a request for study sites to county weed personnel and tribal land managers, focusing on the northwestern United States. Most study sites were selected on the basis of accessibility and availability of local collaborators to participate in the gall collection process. The potential bias of the study site selection process was weighed against logistical constraints of sampling a large number of sites over a wide geographic range, and at locales often distant from transportation corridors situated in rugged terrain or inaccessible due to ownership. The request led to the establishment of study transects (n = 35) located in the state of Washington (n = 3), Oregon (n = 1), Idaho (n = 9), Montana (n = 6), North Dakota (n = 1), and South Dakota (n = 15, Fig. 2.1). *Cirsium arvense* infestation size estimated in hectares, ecoregion type (level III)(EPA, 2013), and habitat type were determined for each study site (Ricketts, 1999). Study site elevation ranged from 1860 m (Site 11, Idaho Falls) to 15 m (Site 1, Silverdale) above sea level. Study sites spanned 15 ecoregions (Ricketts, 1999), six degrees of latitude (42 to 48 ˚N), and habitat types ranging from temperate coniferous forests to xeric shrublands (Table 2.1).

GALL COLLECTION AND REARING

Galls were collected between late summer and the subsequent early spring between 2010/2011 and 2012/2013 along 20 m transects established near the center of CT infestations. Galls were collected during repeated (n = 6) three-minute counts in approximately equal sized visually defined areas surrounding the study transect. All sites were sampled in late summer

when *U. cardui* gall formation was complete and galls were clearly visible, in fall or even in the following very early spring. A collector walked systematically through each of six equal sized collection areas for three minutes, attempting to collect every visible gall. Galls were cut from stems above and below the stem tissue swelling at which point the regular stem diameter resumes. All vegetative material growing from gall surfaces was removed for consistent gall size comparisons. In order to assess parasitism, galls were collected between 8 August and 20 May of the following spring. Galls were collected from Idaho (n = 2371), Montana (n = 1945), South Dakota (n = 1959), North Dakota (n = 92), Oregon (n = 640), and Washington (n = 774).

All galls were stored outdoors at the University of Idaho in Moscow, ID in moisture permeating bags to mimic field temperature and moisture conditions. All galls were brought indoors in spring and allowed to dry in the laboratory at 20° C for 48 h to standardize gall moisture levels and prevent mold build-up within vials.

In order to estimate dry gall weights for all collected galls, 30 galls from each site were dried at 70 °C for 24 h. Galls were weighed to the nearest 0.01 g and estimated dry gall weight (g) was calculated using $y = ax + b$ with $a = 0.727 \pm 0.008$ (mean ± SE) and $b = 0.020 \pm 0.009$ (n = 238, $r^2 = 0.975$).

In order to rear out all insects from *U. cardui* galls they were individually kept in glass vials (29 mm diameter, 65 mm height) covered with fabric. It was important to not let galls dry out because moisture causes callous material that blocks larval tunnels to partially decay and air to diffuse into galls (Lalonde and Shorthouse, 1982) stimulating the development of flies during spring (Redfern, 1983). In order to mimic this process, galls in glass vials were

moistened in frequent (approximately weekly) intervals with droplets of distilled water. Flies were reared at ambient laboratory conditions (20 to 21 °C, LD 12:12).

Insect emergence commenced approximately two weeks following gall relocation into the laboratory. Galls were checked for emerging insect specimen every 48 hrs. until no additional insect had emerged for a minimum period of two weeks. Emerged parasitoids were separated by morphotype and sent to experts for respective taxa for identification. Species were kindly identified by: Dr. Robert Kula (Ichneumonoidea), USDA-ARS Systematic Entomology Laboratory, Washington, D.C.; Dr. Gary Gibson (Chalcidoidea), AAFC Canadian National Collection, Ottawa, Ontario; and Dr. Matthew Buffington (Evanioidea), USDA-ARS Systematic Entomology Laboratory, Washington, D.C.

Following insect emergence, galls were dissected to estimate *U. cardui* larval mortality, and potential parasitoid mortality. For each gall, the number of gall chambers, *U. cardui* pupal casings, dead larvae, and adult flies were recorded. The number of gall chambers was used as an estimate for the original number of second instars present (Frenzel et al., 2000).

Galls with chewing marks, tunnels, excessive deterioration of gall tissue, and those from previous years (identifiable by a darker greyish coloration), were removed from analyses. Galls lacking any insect emergence (n = 3966 of 7002) were also removed from analyses to minimize effects of mortality due to experimental lab conditions (Joseph et al., 2011). Regression was used to predict adult and larval *U. cardui* numbers based upon gall size parameters in order to identify the best predictor variable.

The parasitoid and inquiline gall community of *U. cardui* was compiled by classifying each insect specimen as a parasitoid, inquiline, or facultative gall occupant based on the

location of the insect within a gall. Insect channels were followed back to larval chambers and if leading to a *U. cardui* larval growth chamber, the insect was considered a parasitoid. If emergence tunnels led through gall tissue in an undirected fashion, the insect was classified as inquiline. This method was considered sufficiently robust although it did not allow identification of interactions among non-host insect species.

Parasitoid abundance data for sites that were <60 kms apart from each other were combined (n = 22). Due to the large number of environmentally distinct locations sampled (n = 45), sampling sites were distinguished into three geographical regions for comparisons. These regions were North Dakota and South Dakota (Region D, n = 19), due to the relatively similar elevation and climate. Idaho mountainous field sites (Region M, n = 11) which included study sites in the foothills of or in the Rocky Mountains at generally higher elevations and with more precipitation, and Idaho valley sites (Region V, n = 12), which included study sites largely situated in the Snake River Basin and the Columbia Plateau.

STATISTICAL ANALYSIS

A generalized linear model analysis was used to detect differences in parasitoid distribution and abundance between sites, regions, and years (n = 73), measured through each parasitoid species composition and attack rate, respectively. A similar analysis was used to compare *U. cardui* mortality rates caused by each parasitoid species. In order to estimate the realized caused mortality for each parasitoid species, the proportion of failed gall chambers and the difference between attacked and unattacked galls with gall weight as a fly productivity predictor was measured among galls attacked by the five most abundant parasitoid morphotypes.

Similar to methods used by Joseph et al. (2011), the associations of parasitoid species with gall size were analyzed each year using presence or absence and abundance of each species. Mean gall size utilized by each parasitoid species as well as parasitism rates, for each individual year, were analyzed to confirm consistent parasitoid host selection behavior between years following Joseph et al. (2011). Two-sample t-tests were used to compare parasitized and unattacked gall weights (control). Linear regression analyses were used to assess the effects of parasitoid attack on *U. cardui* gall productivity, measured by the number of adult flies emerged between attacked and unattacked galls, with regard to differing gall size classes used as a predictor of gall productivity.

In order to test whether gall abundance (i.e., number of galls per 3-min count, per site) attracted greater parasitoid attack (i.e., proportion of galls attacked) or influenced parasitoid species richness (i.e., number of parasitoid species per site), ANOVA and linear regressions were used at three gall abundance classes with approximately equal sample sizes.

The proportion of failed gall chambers per site was compared between sites with and without parasitoid presence using a t-test. Parasitoid species interaction effects were accounted for through the use of ANOVA to compare the proportion of *U. cardui* chambers failed among galls that were attacked by multiple parasitoid species, a single parasitoid species, and galls without parasitoid attack (control).

Results

Over the course of the study, on average, 1.16 ± 0.14 (mean ± SE) adult *U. cardui* emerged per gall and 57.82 ± 0.01% (n = 3694) of galls produced adult flies. Although 22.2% (n = 554) of collected galls were older than 12 months (i.e., galls of previous years), *U. cardui*

flies emerged from 13.8% (n = 17) of those galls. The number of *U. cardui* emerged from galls was positively correlated with the following gall size variables: gall volume ($r^2 = 0.495$, $P < 0.001$, $n = 430$), height ($r^2 = 0.246$, $P < 0.001$, $n = 429$), circumference ($r^2 = 0.501$, $P < 0.001$, $n = 430$), and fresh gall weight ($r^2 = 0.573$, $P < 0.001$, $n = 430$). We used fresh gall weight for the following analysis as a predicting variable for the fly emergence numbers because it was also closely correlated with the number of gall chambers within a gall ($r^2 = 0.97$, $P < 0.0001$, $n = 447$).

A total of 22 insect species and one mite were found utilizing *U. cardui* galls in our study. Of these, 17 insects and one mite species were relatively uncommon predators, inquilines, or scavengers (Appendix A). *Urophora cardui* galls were parasitized by five parasitic wasps, to varying degrees (Table 2.3). The most common parasitoid was *Pteromalus* (*Habrocytus*) *elevatus* (Walker), accounting for 78.11 ± 22.50% of all parasitism, followed by *Eurytoma* sp. A (12.13 ± 5.31%), *Eurytoma* sp. B (5.47 ± 3.63%), *Eupelmus (Macroneura) vesicularis* (Retzius) (2.52 ± 0.93%), and *Torymus* sp. (1.78 ± 0.94%, Fig. 2.2). Parasitoid community composition differed between the three study regions in both study years (2011: $\chi^2 = 25.89$, $P \leq 0.001$, $df = 4$, $n = 182$ and 2012: $\chi^2 = 53.41$, $P \leq 0.001$, $df = 8$, $n = 526$, Fig. 2.2 top). Similarly, the rate of *U. cardui* gall parasitism differed between parasitoid species in both study years but attack patterns were similar between regions and between 2011 and 2012 (Fig. 2.2 bottom).

Parasitized galls produced fewer adult flies than unattacked galls in the same size category for *P. elevatus* ($P = 0.001$, $F = 24.32$, $df = 9$, $n = 314$) and *E. vesicularis* ($P = 0.019$, $F = 14.32$, $df = 4$) but there were no differences in gall fly emergence for the *Eurytoma* species (Fig. 2.3). The rate of galls that were parasitized across study sites and years was

11.37 ± 0.01% (n = 6937). Gall attack rates differed greatly among study sites ($P < 0.0001$, χ^2 = 1134.39, df = 70, Fig. 2.4), study regions ($\chi^2 = 19.11$, $P \leq 0.001$, df = 2, Fig. 2.2), years (P = 0.0078, $\chi^2 = 9.70$, df = 2, Fig. 2.2), and species ($P = 0.0001$, $F = 19.01$, n = 53, Fig. 2.4). In 2012, attack rates in the Dakotas region (Region D) was significantly lower compared to the Mountainous (M) and Valley (V) regions ($\chi^2 = 13.61$, $P = 0.0002$, df = 1, Fig. 2.2).

The overall size of unattacked galls collected throughout the course of the study was 0.84 ± 0.03 g (mean ± SE). Attacked galls were 16.03 ± 2.38% smaller than unattacked galls (0.70 ± 0.02 g, $P < 0.001$, $t = 3.84$, df = 1228). The maximum gall weight ratios between healthy and attacked galls were 1:0.79 g (n = 483), 1:0.54 g (n = 2528), and 1:0.49 g (n = 3555), respectively in 2010, 2011, and 2012.

Parasitoids preferentially attacked galls of a certain size range (Fig. 2.5) and that preference was consistent between years. Between parasitoid species, the preferred size gall range was similar ($P = 0.065$, $F = 2.41$, df = 4, Fig. 2.5) with the exception of *Eurytoma* sp. B, which was selecting for galls that were 40.28 ± 13.74% smaller than the average *C. arvense* gall ($P = 0.0034$, $t = 2.93$, df = 6591, Fig. 2.5). In contrast, *E. vesicularis* and *P. elevatus* attacked galls larger than those attacked by *Eurytoma* sp. B ($P \leq 0.05$, Fig. 2.5).

While small parasitized galls (0.0 to 0.75 g) resulted in fewer adult flies than unattacked galls in the same weight class ($P = 0.002$, $t = 3.09$, df = 3549), this difference has only limited consequences because only 0.72 ± 0.02 (mean ± SE) flies emerge from unattacked galls in this size category compared to 0.53 ± 0.04 flies per attacked gall; i.e., a reduction of 26.39 ± 5.56%. There was no difference in the number of emerging *U. cardui* between parasitized and unattacked galls ≥0.70 g, likely because these galls are too big to be attacked by the parasitoids ($P = 0.07$, $t = 1.83$, df = 3006, Fig. 2.4). Galls in this size category

account for 49.84 ± 1.26% (n = 6090) of all galls collected and on average 1.38 ± 0.04 (n = 3311) flies emerged per gall of this size class, further reducing the effect of parasitoid attack on *U. cardui*. There was a positive correlation between the gall abundance at study sites and the number of parasitoid species present (P = 0.014, F = 6.97, df = 27).

At study sites where parasitoids were present (n = 26), a larger proportion of gall chambers produced no adult flies (39.87 ± 2.73%, mean ± SE) compared to sites without parasitoids (n = 20) where 29.22 ± 3.72% of chambers failed (P = 0.02, t = 2.37, df = 123). Similarly, for unattacked galls pooled across all study sites and years, 28.44 ± 2.74% (mean ± SE, n = 25) of chambers did not result in emerging *U. cardui*, compared to 46.58 ± 3.19% (n = 55) for attacked galls (P < 0.001, t = 3.95, df = 78, Fig. 2.5). Over the course of our study, 4.51 ± 1.29% (n = 5063) of gall chambers contained parasitoids. Gall fly larval mortality rates between galls attacked by one or more species of parasitoid were not different, suggesting some degree of hyperparasitism (P = 0.064, F = 2.80, df = 132).

Discussion

PARASITOID COMPLEX

With analogues present in the introduced range, i.e., organisms that are taxonomically and biologically similar and fill functionally the same ecological niche, it is assumed that parasitoid richness of an introduced species should be similar to that of the native range because parasitoid species richness in the introduced range is correlated to the richness of the parasitoid fauna in the native range (Paynter et al., 2010). Thus, we expected that *U. cardui* would be attacked by a similar number of parasitoids in the United States as it is in Europe. In fact, *U. cardui* had a strikingly similar parasitoid complex in the United States as it does in

Europe, consisting of one dominant parasitoid species and three to four additional species that only play minor roles. Another striking observation was that *P. elevatus*, also present in Europe, had switched roles numerically with the *Eurytoma* species and was the dominant parasitoid species for *U. cardui* in the United States. *Urophora cardui* larvae in Europe are primarily attacked by *Eurytoma* species and by a parasitoid in the family Torymidae (Basov, 2002; Schlumprecht, 1989; Zwölfer, 1979). The idiobiont *Eurytoma robusta* (Mayr) accounts for approximately 77% of all parasitism in Europe, while *P. elevatus* accounts for 14%, *Torymus chloromerus* (Walker) for 9%, and *E. serratulae* accounts for less than 5% (Eber and Brandl, 1994; Frenzel et al., 2000; Johannesen and Seitz, 2003b; Redfern, 1983).

Pteromalus elevatus is found in Europe, northern Asia, and eastern Canada to Saskatchewan (Hoebeke and Wheeler Jr, 1996). *Pteromalus elevatus* may have been accidentally introduced to North America in 1923 with the gall-forming tephritid, *Urophora jaceana* (Hering), also accidently introduced from Europe with *Centaurea nigra* (L.) into Nova Scotia (Graham, 1969; Shewell, 1961), or with releases of *U. cardui* in eastern Canada (Hoebeke and Wheeler Jr, 1996). *Urophora cardui* is one of six known *Urophora* hosts of *P. elevatus* (Noyes, 2012) and attack of the gall fly was previously considered low in Canada with 3% of attacked larvae from *C. arvense* galls in a study conducted in New Brunswick (Peschken et al., 1982). An individual *P. elevatus* larva matures in each host larvae and can attack larvae of other present parasitoid species (Jones et al., 1996; Vanbergen et al., 2006; Williams et al., 2001). *Pteromalus elevatus* is multivoltine but was nonetheless considered of little threat to biological control, except for possible minor effects it may have on *U. cardui* (Peschken et al., 1982).

Eurytomidae can be phytophagous and/or entomophagous depending on the genus. The divisions among genera can be unclear and the boundaries of subfamilies are a matter of speculation (Gates, 2001). The two parasitoid species found in our study could not be determined beyond the genus level due to a current lack of experts for this group in the Nearctic (M. Gates, personal communication). *Eurytoma* is the most common genus in the family (DiGiulio, 1997; Gibson et al., 1997). Within each host puparium, a single *Eurytoma* sp. larva overwinters (Basov, 2002; Claridge, 1961). Species in this genus have been previously reported parasitizing *U. cardui* including: *E. compressa* (F.), *E. robusta* (Mayr), and *E. serratulae* (Latr.) (Noyes, 2012).

Eupelmus vesicularis is found in North Africa, the Middle East, Asia Minor, Europe, and North America (Gibson, 1990). Typically, *E. vesicularis* only persists as far south as 36 °N. The parasitoid was unintentionally introduced from Europe with some of the earliest settlers, most likely in contaminated straw. It was first reported in Pennsylvania in 1915 (Gahan, 1933; Krombein et al., 1979; Peck, 1963). *Eupelmus vesicularis* is one of the most polyphagous chalcidoids known with more than 130 host insects recorded (Gibson, 1990). The fact that we found *E. vesicularis* attacking *U. cardui* is not unusual as most hosts of the parasitoid are gall formers or stem-miners (Gibson et al., 2006). This is, however the first record for the species on *U. cardui* in North America.

The life cycle and behavior of Torymidae have not been well studied (Basov, 2002) and they utilize host insects within several insect orders, including gall flies (Sellenschlo and Wall, 1984). Many Torymidae are primary parasitoids but some are facultative multi-parasitic (two or more parasitoids attacking one host larva), resulting in complex relationships among different parasitoid species and the host insect (Weis, 1982). Torymidae species attack a

single larvae at the time of oviposition (Askew, 1980). Some Torymidae are known to select small host galls (0.5 to 1.5 mm in diameter) or those that mature late during summer and are still small at the time the parasitoids are active, due to short ovipositor length (Joseph et al., 2011).

PARASITOID ATTACK RATES

Although *U. cardui* galls attacked by two of the parasitoid species resulted in fewer emerging flies, the difference was small and in addition the respective parasitoid species are only able to attack smaller *U. cardui* galls, further reducing the effect. The other parasitoids found to attack *U. cardui* in our study had no effect on *U. cardui* larval survival. There was no difference in overall larval mortality among galls attacked by the parasitoid species. This is not surprising considering that *T. chloromerus* and *P. elevatus* had also only marginal influence on *U. cardui* larval mortality in the native range with <1% larval mortality in northeastern Bavaria (Frenzel et al., 2000).

Parasitoid attack rates of *U. cardui* galls differed considerably among sites, regions, years, and parasitoid species. This result is not suggestive of a universally and slowly growing parasitoid population that might exact control over the host insect population. Instead, *U. cardui* may be escaping predation and parasitism due to its meta-population dynamic (Eber and Brandl, 1994; Eber and Brandl, 1996; Zwölfer, 1979) within fragmented host plant populations (Kruess and Tscharntke, 1994) that occur primarily in ephemeral habitats (Juliano, 2007; Wissinger, 1997). Similar levels of parasitism variability are also observed in the native range. Mean rates of parasitism in Europe ranged from 23.3% in 1988 to 78.7% in 1991 (Eber and Brandl, 1994; Eber and Brandl, 1996).

The overall parasitism rate reported here of 11.37% is well below rates assessed across Germany and Denmark with 27.04% (Johannesen and Seitz, 2003a). Given that the parasitism rates in the United States are much lower than in the native range, it is highly unlikely that parasitoids are a local key factor in *U. cardui* population dynamics or compromise the efficacy of *U. cardui* in the United States, as is the case in Europe (Zwölfer et al., 2007).

Parasitism rates of insects tend to be lower in their introduced ranges compared to the native range, in part because isolated habitat fragments tend to reduce parasitoid diversity and parasitism rates compared to contiguous habitats (Tscharntke and Kruess, 1999). In addition, host plant, host insect, and parasitoid synchronization are important factors for the success of parasitoid attack and this synchronization can easily be asymmetrically affected by changing climatic conditions in a given year (Basov, 2002; Schlumprecht, 1990). A lack of synchronization may contribute to lower observed parasitoid abundances in the geographically and climatically much more variable introduced range in the United States, although we did not measure this. *Urophora cardui* may also escape from parasitoid attack through other mechanisms including but not limited to escape in space and/or time, exploitation of habitat heterogeneity, and large clutch sizes (Johannesen and Seitz, 2003b). Because of the diversity of parasitoids found to attack *U. cardui* in our study and the similarity of the parasitoid complex to that found in its native range, we do not however believe that the gall fly benefits from enemy release in the United States (Keane and Crawley, 2002).

GALL SIZE AND ABUNDANCE EFFECTS

In our study, attacked galls were smaller than unattacked galls and among the attacked galls, those attacked by certain parasitoid species were consistently smaller than galls attacked by other species. The composition and abundance of the parasitoid assemblage can be a function of gall traits, such as size and toughness, assuming that parasitoids exploit galls with a specific set of traits and that the preferences can be used as a predictor of the parasitoid community (Joseph et al., 2011). Although parasitoid species may also attempt to attack galls that are larger than those they can successfully attack at times when parasitism rates are very high to escape competition (Johannesen and Seitz, 2003b), there is a general negative correlation between parasitism rates and gall size. This relationship is due to the following factors: first, increasing gall size increases parasitoid handling time compromising its reproductive fitness; second, larger galls offer the gall-inducing insect protection because of thicker impenetrable gall tissues, or ovipositor length restriction (Joseph et al., 2011; Weis, 1982); and third, gall-inducing insects attacked by ectoparasitoids may not be able to produce large galls because of the premature death of the host larvae (Johannesen and Seitz, 2003b; Joseph et al., 2011; Zwölfer and Arnold-Rinehart, 1994).

We found that large galls in our study were attacked less by parasitoids, but also that when attacked, it did not impair *U. cardui* larvae in those galls functionally, i.e., there was no difference in gall fly mortality between attacked and unattacked galls in the respective size class. The largest galls found in our and other studies, weighted nearly 13 g, and contained up to 21 chambers (Basov, 2002; Weis, 1983). Nonetheless, the highest survival rate of *U. cardui* larvae into adulthood was reported for average sized galls (Weis, 1983), which may be due to differing selection pressures. Small galls are subject to higher potential parasitoid attack but

larger galls may be preferably predated upon. Gall mass can be in conflicting selection pressure through parasitoids that select smaller galls and predators that specially utilize larger galls (Weis and Kapelinski, 1994).

Although there was a tendency of higher parasitoid attack rates at sites with higher gall abundance, parasitoid attack is generally not considered to be a function of host insect aggregation because parasitoids are easily oversaturated with potential insect hosts and are then constricted by their own handling and oviposition time (Papaj and Alonso-Pimentel, 1996). We also observed an increase in parasitoid richness with increasing host gall abundance. Limitation of a host population may be unaltered by the occurrence of several generalist parasitoid species whose utilized resources entirely intersect (Finke and Snyder, 2008). Some parasitoid species, including *E. robusta*, lay eggs regardless of the presence and distribution pattern of other parasitoids (Johannesen and Seitz, 2003b). Because *U. cardui* parasitoids in the introduced range are generalists, increasing parasitoid richness may not result in increasing parasitism pressure on *U. cardui*.

PARASITISM EFFECTS ON *U. CARDUI* POPULATIONS

In the native range, studies found parasitism rates of *U. cardui* gall chambers between 30 and 33% (Basov, 2002; Zwölfer et al., 2007), much higher than the 4.51% parasitoid cell composition rate observed in our study. Attacked galls' larval mortality rates were nearly 20% higher than that of healthy galls. However, at the sub-population level, sites with parasitoid presence only had a 10% increase in larval mortality compared to sites lacking parasitoids. Our observed lack of differing larval mortality between galls attacked by one or more species

suggests interspecific hyperparasitism, resulting in decreased parasitism pressure on *U. cardui* (Basov, 2002; Ghahari and Huang, 2012).

In small subpopulations of *U. cardui*, parasitism can affect colonization rate and lead to high mortality rates and local extinction (Eber and Brandl, 1997; Freese, 1997; Frenzel et al., 2000; Schlumprecht, 1990; Zwölfer, 1979; Zwölfer and Arnold-Rinehart, 1993). However, flies in large populations can show equilibrium with parasitoids for some time (Schlumprecht, 1990) and are not limited by parasitoid mortality (Johannesen and Seitz, 2003a). Parasitism rates observed in our study indicate that 2.10% (11.48% maximum per site) of *U. cardui* larvae in the United States die due to parasitism. Parasitoids that kill fewer than 40% of a host insect population, rarely control the host (Barlow et al., 2003; Hawkins et al., 1993).

Our results indicate that *U. cardui* does not suffer from high parasitism pressure in the introduced range. In a study across Germany and Denmark, parasitism rates for *U. cardui* were 69.9% but declined to <25% the subsequent three study years with no apparent effect on *U. cardui* populations (Johannesen and Seitz, 2003b). Consequently, we would not expect that *U. cardui* populations in the United States are impaired by parasitoids to any degree, which theoretically should allow the gall fly to have a greater effect on *C. arvense* (Keane and Crawley, 2002; Paynter et al., 2010). According to the enemy release hypothesis (Elton, 1958; Keane and Crawley, 2002), *U. cardui* could be considered to at least escape some of its natural enemy pressure, i.e., parasitoid attack rates in the United States and thus, populations should be able to grow larger than they would in its native range (Paynter et al., 2010). On the other hand, we found a complex of parasitoids that is comparable with that of *U. cardui* in its native range, with one of the main parasitoids species occurring in both ranges. Thus, while

the attack rates in the United States are lower than in its native European range, functionally, *U. cardui* is not escaping natural enemies in the United States.

Finally, we do not expect the parasitoid pressure on *U. cardui* to change dramatically in the western United States. It is expected that a visually apparent gall-forming host insect accumulates parasitoids over time, including niche-specific native parasitoids that would switch hosts to attack *U. cardui* (Lawton, 1985; Paynter et al., 2010). However, susceptible weed biological control agents, especially visually apparent gall-formers, rapidly accumulate parasitoids and time plays a less important role in further increasing that parasitoid diversity (Hill and Hulley, 1995; Paynter et al., 2010). Therefore, we conclude that it is unlikely that *U. cardui* will acquire a large number of additional parasitoids or that parasitism pressure will further increase in the western United States.

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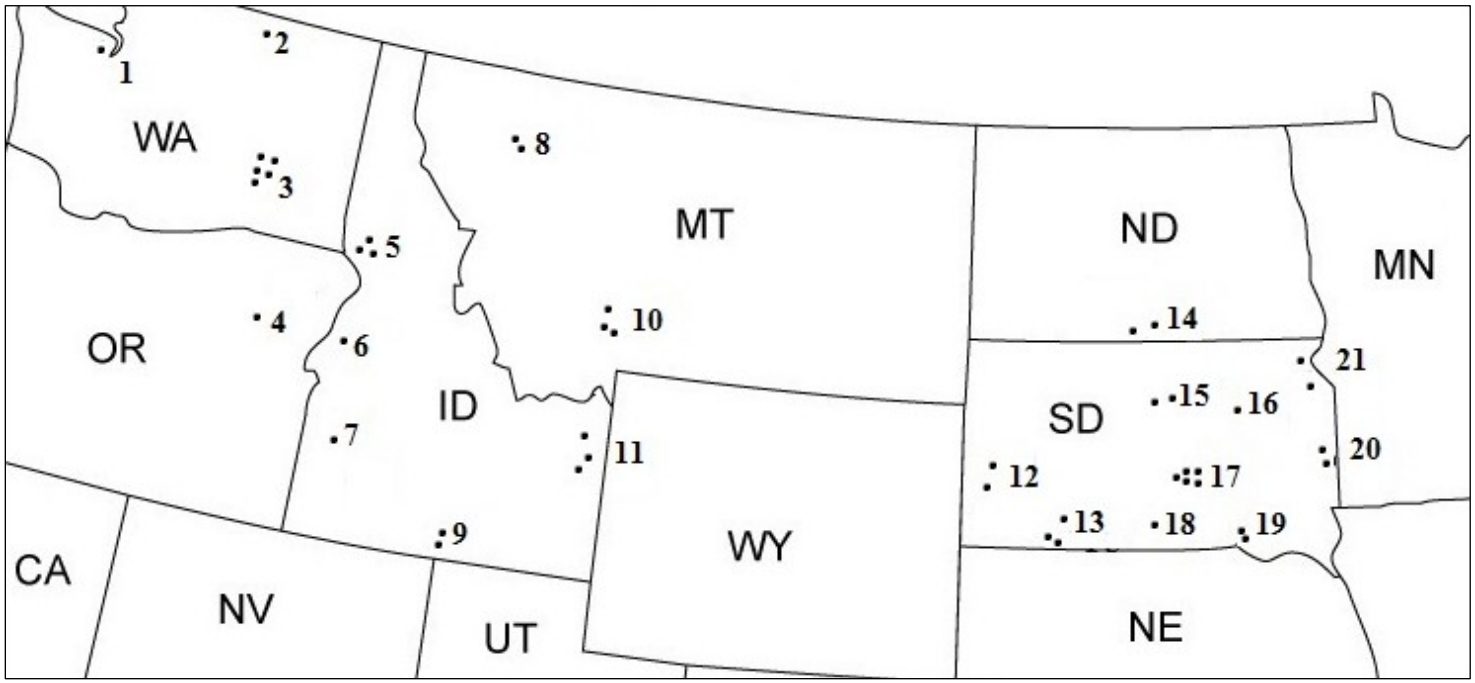

Figure 2.1 Study transect locations in northwestern and northern plains states of the United States. See Table 2.1 for study site information.

Table 2.1 Populations of *Urophora cardui* included in study.

Map No.	Site name	State	Coordinates (NAD83)		Elevation (m)	Patch size (ha)	Land use	Ecoregion*	Habitat type
1	Silverdale	WA	N 47° 40′ 12.0″	W 112° 41′ 15.0″	15	—	Recreational trail	Strait of Georgia/ Puget Lowland	Temperate coniferous forests
2	Riverside	WA	N 48° 31′ 08.0″	W 119° 22′ 38.0″	756	—	Rangeland	Columbia Mountains/ Northern Rockies	Temperate grasslands, savannas, and shrublands
3	Connell	WA	N 46° 31′ 54.0″	W 119° 15′ 31.0″	131	—	Roadside drainage	Columbia Plateau	Deserts and xeric shrublands
4	La Grande	OR	N 45° 15′ 23.8″	W 118° 01′ 54.5″	827	—	Wetland WMA	Blue Mountains	Temperate coniferous forests
5	Troy	ID	N 46° 45′ 16.9″	W 116° 44′ 21.0″	851	0.03	Forested foothills	Columbia Mountains/ Northern Rockies	Temperate coniferous forests
5	Idler's Rest	ID	N 46° 48′ 07.2″	W 116° 57′ 02.7″	902	0.05	Recreational trail	Columbia Mountains/ Northern Rockies	Temperate coniferous forests
5	Moscow	ID	N 46° 43′ 14.3″	W 116° 59′ 10.1″	791	0.01	Urban drainage	Columbia Plateau	Temperate coniferous forests
6	Adams Co.	ID	N 45° 00′ 05.6″	W 116° 17′ 48.9″	1,188	0.81	Urban rec. area	Idaho Batholith	Temperate coniferous forests
7	Nampa	ID	N 43° 33′ 42.0″	W 116° 39′ 11.4″	753	0.15	Roadside marsh	Snake River Plain	Deserts and xeric shrublands
8	Kalispell	MT	N 48° 03′ 57.1″	W 114° 07′ 35.2″	884	—	Lake shoreline	Columbia Mountains/ Northern Rockies	Temperate coniferous forests
8	Wise	MT	N 48° 10′ 03.3″	W 114° 10′ 21.2″	884	—	River shoreline	Columbia Mountains/ Northern Rockies	Temperate coniferous forests

8	Moon	MT	N 48° 09′ 09.9″	W 114° 13′ 31.2″	885	—	Cropland	Columbia Mountains/ Northern Rockies	Temperate coniferous forests
9	Burley	ID	N 42° 16′ 30.3″	W 114° 02′ 12.0″	1,499	0.02	Sagebrush steppe riparian	Northern Basin and Range	Deserts and xeric shrublands
10	Toston	MT	N 46° 09′ 25.0″	W 111° 29′ 37.0″	1,204	—	Pasture	Middle Rockies	Temperate grasslands, savannas, and shrublands
10	19th Street	MT	N 45° 41′ 50.0″	W 111° 03′ 31.0″	1,447	—	Cropland	Middle Rockies	Temperate grasslands, savannas, and shrublands
10	MSU	MT	N 45° 40′ 09.0″	W 111° 03′ 13.0″	1,489	—	Urban drainage	Middle Rockies	Temperate grasslands, savannas, and shrublands
11	St. Anthony	ID	N 44° 00′ 34.1″	W 111° 36′ 23.1″	1,539	—	Wetland WMA	Snake River Plain	Deserts and xeric shrublands
11	Idaho Falls	ID	N 43° 23′ 41.3″	W 111° 43′ 23.9″	1,860	—	Rangeland drainage	Snake River Plain	Deserts and xeric shrublands
11	Ririe	ID	N 43° 35′ 37.2″	W 111° 29′ 16.8″	1,553	—	Cropland riparian	Snake River Plain	Deserts and xeric shrublands
12	Burnt Fork	SD	N 43° 57′ 39.8″	W 103° 36′ 10.6″	1,594	—	Roadway riparian	Middle Rockies	Temperate coniferous forests
12	Bear	SD	N 44° 28′ 09.4″	W 103° 26′ 50.7″	966	—	Wildlife refuge drainage	Middle Rockies	Temperate coniferous forests
13	Pine Ridge	SD	N 43° 07′ 07.4″	W 102° 09′ 39.7″	1,037	0.06	Cropland drainage	High Plains	Temperate grasslands, savannas, and shrublands

14	Standing Rock	ND	N 46° 13′ 47.1″	W 100° 42′ 40.4″	519	0.04	Riparian	Northwestern Great Plains	Temperate grasslands, savannas, and shrublands
15	Potter	SD	N 45° 09′ 19.3″	W 099° 40′ 29.4″	579	—	Cropland	Northwestern Glaciated Plains	Temperate grasslands, savannas, and shrublands
15	Cheyenne River	SD	N 45° 02′ 11.1″	W 100° 28′ 52.8″	492	—	Marsh	Northwestern Great Plains	Temperate grasslands, savannas, and shrublands
16	Watertown	SD	N 44° 53′ 35.9″	W 098° 29′ 57.7″	391	—	Cropland riparian	Aspen Parkland/ Northern Glaciated Plains	Temperate grasslands, savannas, and shrublands
17	Crow Creek	SD	N 44° 06′ 24.2″	W 099° 28′ 42.0″	453	0.2	Buffalo rangeland	Northwestern Great Plains	Temperate grasslands, savannas, and shrublands
17	Lower Brule	SD	N 44° 04′ 24.0″	W 099° 35′ 28.0″	467	0.12	Rangeland	Northwestern Great Plains	Temperate grasslands, savannas, and shrublands
17	Red Green	SD	N 44° 04′ 58.0″	W 099° 34′ 58.9″	436	—	Recreation area	Northwestern Great Plains	Temperate grasslands, savannas, and shrublands
18	Rosebud	SD	N 43° 21′ 02.5″	W 100° 24′ 14.8″	711	0.03	Rangeland	Northwestern Great Plains	Temperate grasslands, savannas, and shrublands
19	Yankton	SD	N 43° 06′ 47.8″	W 098° 27′ 55.4″	499	0.09	Cropland	Northwestern Glaciated Plains	Temperate grasslands, savannas, and shrublands
20	Flandreau	SD	N 44° 03′ 42.0″	W 096° 33′ 05.4″	468	—	Riparian	Western Corn Belt Plains	Temperate grasslands, savannas, and shrublands

20	Brookings	SD	N 44° 19′ 21.9″	W 096° 46′ 16.9″	499	—	Hedgerow	Aspen Parkland/ Northern Glaciated Plains	Temperate grasslands, savannas, and shrublands
21	Codington	SD	N 44° 55′ 59.4″	W 097° 06′ 23.3″	539	—	Parking lot	Aspen Parkland/ Northern Glaciated Plains	Temperate grasslands, savannas, and shrublands
21	Sisseton	SD	N 45° 30′ 54.8″	W 096° 55′ 38.7″	367	0.07	Cropland	Northern Glaciated Plains	Temperate grasslands, savannas, and shrublands

* Ecoregions classification (Level III) (EPA, 2013).

Table 2.2 Parasitoid Hymenopteran species found to attack galls of *Urophora cardui.*

Species	Order	Family	Biology	State present
Pteromalus elevatus (Walker)	Hymenoptera	Pteromalidae	Palearctic. Accidental introduction into North America. Primarily parasitizing larvae of gall forming tephritids in flower-heads of composites. Not previously reported in contiguous United States. Previously reported to attack *U. cardui* in Canada and Europe [a]	ID, OR, MT, WA, SD
Eurytoma sp. A	Hymenoptera	Eurytomidae	Cosmopolitan. Polyphagous. Can be found in galls of many insects as a primary or secondary parasitoid, especially tephritid galls on Asteraceae. Congeners previously reported to attack *U. cardui.*[b]	ID, OR, MT, WA, SD
Eurytoma sp. B	Hymenoptera	Eurytomidae	See above for *Eurytoma* sp. A.	ID, OR,MT, SD
Eupelmus vesicularis (Retzius)	Hymenoptera	Eupelmidae	Cosmopolitan. Polyphagous primary or secondary parasitoid of concealed larvae in 20 insect families from 6 orders. Accidentally introduced into United States in 1915. Congeners previously reported to attack *U. cardui*.[c] *U. cardui* is, however, a new host record for *E. vesicularis.*	ID, OR, MT, WA, SD
Torymus sp.	Hymenoptera	Torymidae	Polyphagous. Common idiobiontic ectoparasites of gall forming insect larvae, including Tephritidae. Some are hyperparasitoids on *Eurytoma* spp. Genus previously reported in *U. cardui* galls. First reported in United States in 1958.[d]	ID, OR, MT, WA, SD

[a], Graham (1969); Noyes (2012); [b], Noyes (2012); [c], Gahan (1933); Krombein et al. (1979); Noyes (2012); Peck (1963); [d], Askew (1980); Noyes (2012); Sellenschlo and Wall (1984).

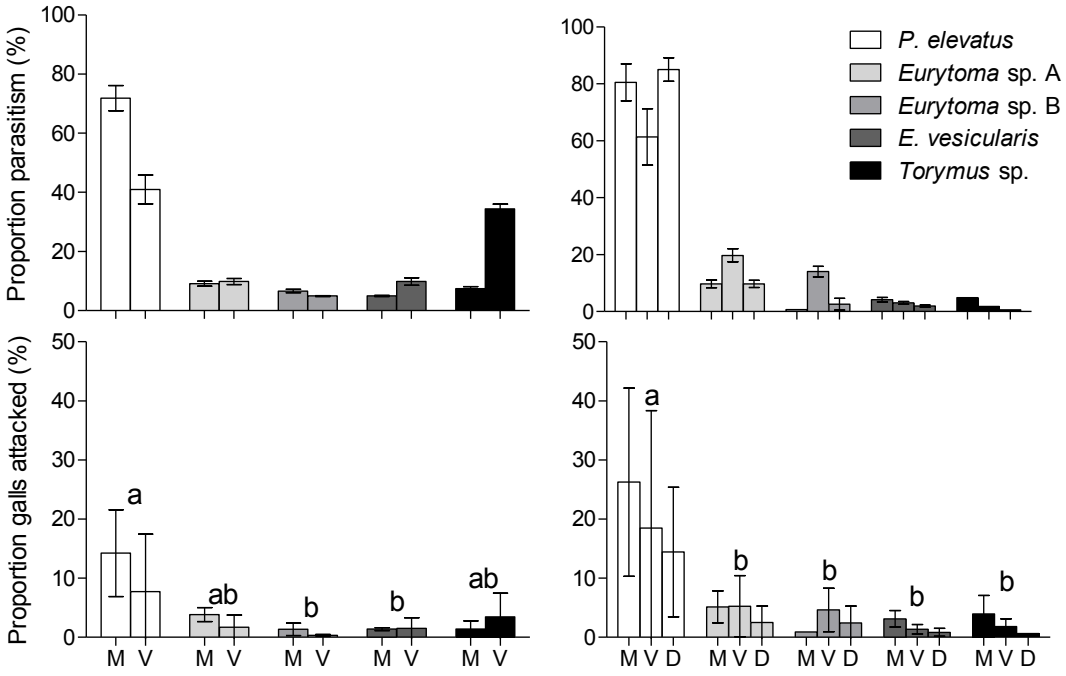

Figure 2.2 Proportion parasitism by parasitoid species and study region (top graphs) and proportion of *U. cardui* galls attacked per site by parasitoid species (bottom graphs) in 2011 (left) and 2012 (right). M, mountainous region, V, valley region, and D, North and South Dakota region. 2011 lacks data for D region due to technical problems with the gall rearing method (2011, $n = 182$; 2012, $n = 526$). Different letters above data indicate significant differences between species attack rates ($P < 0.05$).

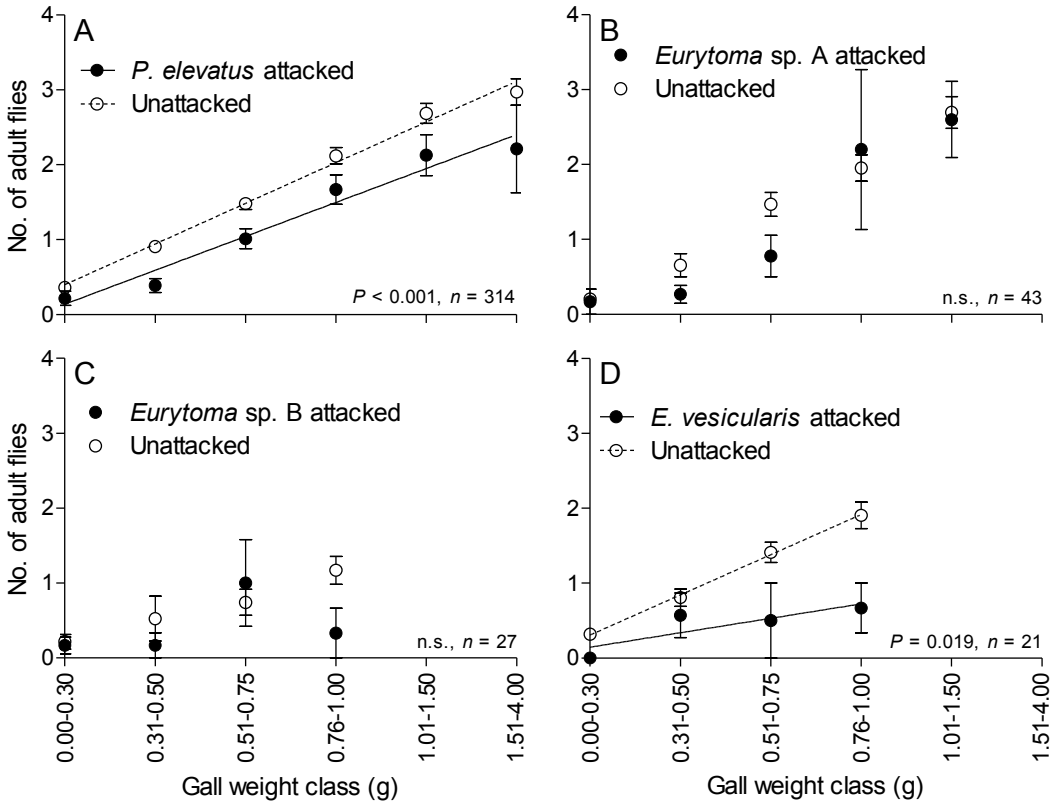

Figure 2.3 Impact of parasitism on the number of emerging *Urophora cardui* flies from galls in different gall weight classes; n.s., not significant.

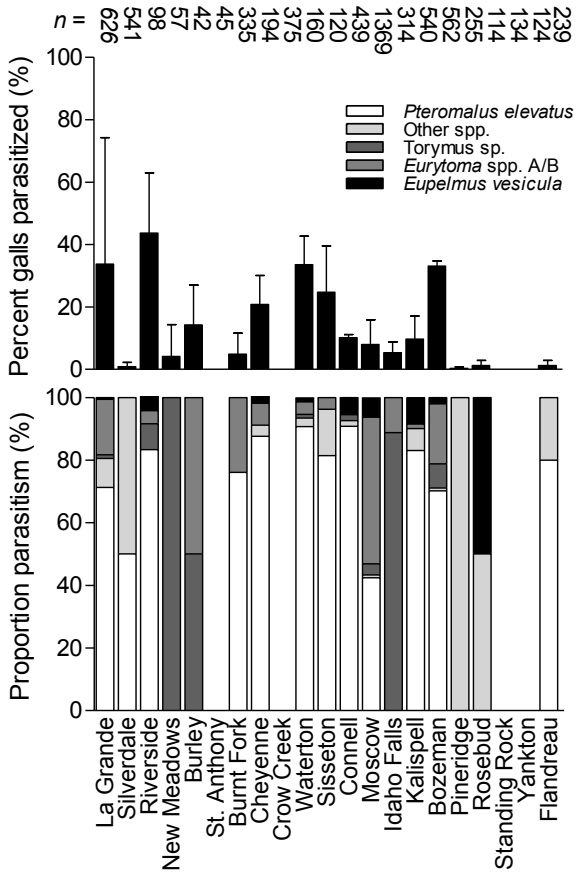

Figure 2.4 Percentage of *U. cardui* galls parasitized at study sites (top graph) and proportion parasitism by Hymenopteran parasitoid species (bottom graph).

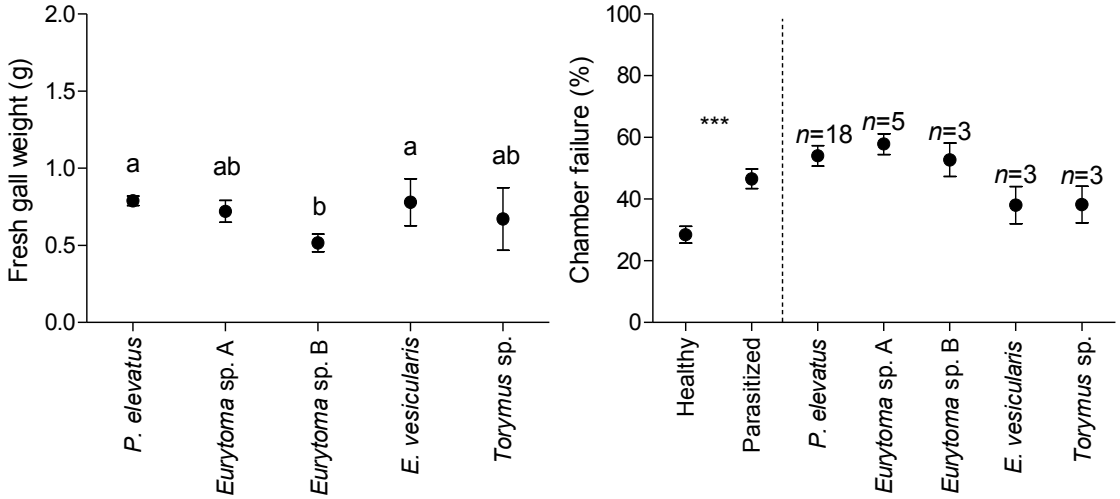

Figure 2.5 Weight (mean ± SE) of *U. cardui* galls parasitized by different parasitoid species (left) and proportion of gall chambers per gall failing to result in fly emergence (right). Different letters above data indicate significant differences between gall weights (Tukey's multiple comparison test after analysis of variance, $n = 492$, $P < 0.01$). ***$P < 0.001$.

CHAPTER 3: POST-RELEASE EFFICACY ASSESSMENT OF *UROPHORA CARDUI* AND *HADROPLONTUS LITURA*, BIOLOGICAL CONTROL AGENTS OF THE INVASIVE *CIRSIUM ARVENSE*

Abstract

Classical biological control of weeds has been used for almost 50 years to control *Cirsium arvense* (Canada thistle) in the United States. However, there are few studies that have assessed the efficacy of the two approved biological control agents, the stem-galling tephritid fly *Urophora cardui* (L.) and the stem-mining weevil *Hadroplontus litura* (F.) on *C. arvense* in the field. We set-up 87 permanent transects, following a standardized impact monitoring protocol (SIMP), at *C. arvense* infestations in Idaho (n = 44), Utah (n = 8), Wyoming (n = 4), North Dakota (n = 5), and South Dakota (n = 26) to monitor *C. arvense* and biological control agent populations for a five year period between 2008 and 2012. We measured biological control agent abundance, plant cover in five broad vegetation categories, and *C. arvense* stem density for each transect. We setup groups of four transects at least 1 km distant from each other and randomly assigned one of four treatments to each transect: release of *U. cardui* flies, release of *H. litura* weevils, release of both insects, or no insect releases. For our analysis we also included biotic and abiotic site variables to parameterize a discrete population model aiming to explain changes in *C. arvense* stem density between years. While *U. cardui* and *H. litura* established and were widely distributed, their abundances varied greatly and were generally low. The weevil and the fly lacked an effect on *C. arvense* stem density change. *Cirsium arvense* stem density during the previous year negatively affected the *C. arvense* growth rate, indicating negative plant-feedback, likely due to intraspecific competition. Perennial grass cover also had a negative effect on the *C. arvense* growth rate,

providing potential opportunities for restoration strategies. In our study, *U. cardui* and *H. litura* provided no control for *C. arvense*.

Introduction

Exotic invasive plants have large impacts on agricultural production and biological diversity worldwide (Levine et al., 2003; Pysek et al., 2011). They gain competitive superiority over native plant species in areas of introduction (Levine et al., 2003), but the mechanisms that allow exotic plants to successfully invade recipient plant communities and agro-ecosystems are still not well understood. Some evidence suggests that increased resource availability can be linked to plant invasions (Davis et al., 2000; Shea and Chesson, 2002). Alternatively, reduced or absent complexes of specialist natural enemies in the recipient communities of invasive plants are thought to contribute to invasion success, as postulated in the enemy release hypothesis (Elton, 1958; Keane and Crawley, 2002; Mitchell and Power, 2003), and both processes may act synergistically (Blumenthal et al., 2009).

Classical biological control of weeds, the introduction of host-specific herbivore natural enemies from the native range of the invasive plant to permanently reduce its abundance and density in the introduced range, is a widely used integrated pest management practice based on the enemy release hypothesis (Keane and Crawley, 2002; Van Driesche et al., 2009). Field studies based on natural enemy exclusion experiments in the invaded range have provided some of the strongest support for the enemy release hypothesis and thus, the ability of specialist herbivory to negatively affect invasive plant population dynamics (McEvoy et al., 1991). However, there are only few studies that have attempted to assess how classical biological weed control, resource availability and other feedback mechanisms, may

interact, and what the resulting combined effects on invasive plant dynamics may be (Schooler et al., 2011; Weed and Schwarzländer, 2014).

Documenting the ecological and economic benefits or effectiveness of biological weed control programs (Barratt et al., 2010; Blossey, 1999), reasons for their failures (Bacher et al., 1999), or unintentional non-target effects (Louda and O'Brien, 2002) has become increasingly important to justify the investment of mostly public resources and to improve the success probabilities of future programs (Greer and Sheppard, 1990). As a consequence, the number of post-release weed biocontrol assessments has increased substantially (Crawley, 1989; Grevstad, 2006; Hough-Goldstein et al., 2008; McEvoy et al., 1991; Myers et al., 2009; Schooler et al., 2011; Syrett et al., 2000) but the number of assessments is still small compared to the number of ongoing biological weed control efforts. There are also, few studies that have tried to mechanistically link biocontrol herbivory with plant population dynamics or consider simultaneously acting processes, such as resource availability or feedback mechanisms (Schooler et al., 2011). Overall, recent reviews on biological weed control programs concluded that biological control impact on invasive plant population biology is often varied and uncertain (Clewley et al., 2012; Stephens et al., 2013).

The herbaceous invasive plant *Cirsium arvense* (L.) Scop. (Asteraceae) (Canada thistle, California thistle, Creeping thistle, hereafter CT) was introduced to North America with the first European settlers in the 1600s, and later, likely again from Eastern Europe into the United States prairie (Guggisberg et al., 2012). *Cirsium arvense* is considered one of the worst invasive plants of agro-ecosystems and natural areas worldwide with severe economic losses to crop yields, rangeland, and pasture productivity (Hartley and James, 1979; Mitchell

and Abernethy, 1993). Attempts to control CT date back more than a hundred years (Burrhill, 1890), but management of CT has thus far been generally unsuccessful (Cripps et al., 2011).

Cirsium arvense success as an invasive in North America (Pritekel et al., 2006) may be, in-part, due to release from specialist natural enemies found in the native Eurasian range (Keane and Crawley, 2002). The plant is considered a difficult target for classical biological control of weeds due to a number of traits, including the fact that it is also a major weed in its native range (Schroeder et al., 1993). Nonetheless, classical biological control of CT has been attempted in the United States since 1966 (Julien and Griffiths, 1998), probably because the benefits of a potentially successful biocontrol program against the economically significant plant would offset the relatively high probability of failure (Page and Lacey, 2006; Paynter et al., 2012). Not surprisingly, after implementing biological control for more than four decades, there is little indication for successful control of the weed elsewhere (Fowler et al., 2000; McClay et al., 2002).

Three biological control agents have deliberately been introduced into the U.S. for the control of CT but only two established: the stem gall fly *Urophora cardui* (L.) (Diptera, Tephritidae), first introduced in 1977, and the stem-mining weevil *Hadroplontus litura* (F.) (Coleoptera, Curculionidae), first introduced in 1971 (Julien and Griffiths, 1998). The post-release impact of *U. cardui* (Peschken et al., 1982), *H. litura* (Hein and Wilson, 2004; Larson et al., 2005; Peschken and Wilkinson, 1981; Reed et al., 2006; Rees, 1990), and both species combined (Markin and Larson, 2013; Peschken and Derby, 1992) has been assessed previously in the U.S. and elsewhere. However, most of these studies were conducted in controlled settings, at small spatial scales, or only for short periods of time. A comprehensive

analysis on limitations of either species to successfully control CT across the countries where the insects have been released has been provided by Cripps et al. (2011).

Because of renewed interest from land managers to implement biological control for CT in the western U.S. using *U. cardui* and *H. litura*, we wanted to expand on previous studies and assess the impact of *U. cardui* and *H. litura* in the field, at a larger spatial scale, over a longer time period. We also wanted to assess effects of simultaneously acting other endogenous and exogenous factors on CT and their potential interactions with biological weed control. Our specific objectives were to 1) assess *U. cardui* and *H. litura* establishment rates and abundance following release, 2) describe herbivory effects on individual CT ramets, and 3) assess effects and potential interactions of biocontrol, and other exogenous and endogenous factors, on CT ramet density fluctuations between years.

Materials and methods

STUDY SYSTEM

Cirsium arvense is a perennial herbaceous plant native to south-eastern Europe and the eastern Mediterranean (Donald, 1990; Moore, 1975; Morishita et al., 1999; Skinner et al., 2009). It is considered one of the "world's worst weeds" and is distributed throughout temperate regions of the globe (Friedli and Bacher, 2001b; Holm et al., 1977; Moore, 1975; Pritekel et al., 2006; Tiley, 2010).

Seedlings of CT produce basal rosettes that bolt in late spring and die back in fall (Gange et al., 2012). *Cirsium arvense* is a clonal and dioecious plant that can produce several hundred ramets with stems up to 220 cm in height. Stems have spiny leaves that alternate, branch freely, and produce up to 1500 seeds (Hay, 1937). Seed dispersal can be up to one

kilometer from the parent plant, or more, if carried away by wind or flowing in water (Bostock and Benton, 1979; Nuzzo, 1997). Plants also reproduce vegetatively through horizontal subterranean shoots that generate numerous flowering ramets (Bourdot et al., 1995).

Cirsium arvense grows in temperate environments where soils are disturbed (e.g., eroded, previously flooded, or heavily grazed) but performs poorly in undisturbed pastures (Bossard et al., 2000; Evans, 1984; Zouhar, 2001). The plant prefers meadows, riparian areas and habitats with access to moisture. Rangeland or crops invaded by *C. arvense* (Donald, 1990; Jacobs et al., 2006) result in economic losses (Hartley and James, 1979; McLennan et al., 1991; Mitchell and Abernethy, 1993; Moyer et al., 1991; O'Sullivan et al., 1982).

The gall-forming fruit fly, *U. cardui*, is native to central Europe (Seitz and Komma, 1984) and was first introduced for the biological control of CT into the U.S. in 1977 (Julien and Griffiths, 1998; Lalonde and Shorthouse, 1984; Peschken et al., 1997). *Urophora cardui* is host specific to *C. arvense* and, to date, there are no reports on non-target plant use (Zwölfer and Harris, 1971). The flies lay eggs in the upper parts of nearly mature stems that result in multi-chambered galls in which up to 14 fly larvae develop and pupate. Univoltine flies overwinter and emerge from galls the following spring.

Urophora cardui galls are considered a plant metabolic sink which may result in reduced CT fitness and competitive ability (Harris and Shorthouse, 1996; Thibodeau, 1985). Although *U. cardui* is well established in North America and locally abundant (McClay et al., 2002), there is no or little measureable impact in the field (Fay et al., 1996; Forsyth and Watson, 1984; Harris and Shorthouse, 1996; Julien, 1987; Peschken and Derby, 1992).

The stem-mining weevil, *H. litura*, is native to Western Europe and was first introduced into the U.S. in 1971 (Julien and Griffiths, 1998; Zwölfer and Harris, 1966). *Hadroplontus litura* is a univoltine weevil that overwinters in soil litter and emerges in spring, to feed on CT rosette foliage. Females lay eggs in the midrib of leaves and hatching larvae mine into stems and consume parenchymatic pith (Peschken and Wilkinson, 1981). Mature larvae mine toward the root crown and exit the stems during summer to pupate in the soil (Rees, 1990).

Hadroplontus litura is established throughout North America but is considered uncommon and limited by a low dispersal rate and reproductive ability (Peschken and Beecher, 1973). The larvae chew holes in CT stems that may facilitate secondary infections by pathogens, but pathogens are generally not considered promising control organisms for CT (Müller et al., 2011). Studies on the efficacy of *H. litura* have shown little impact of the weevil on CT in the field, likely due to the fact that *H. litura* larvae feed exclusively on nonessential plant tissues and/or the relatively low abundance of the insect (Burns et al., 2013; Peschken and Wilkinson, 1981; Reed et al., 2006; Zwölfer and Harris, 1966). Nonetheless, land manager demand for *U. cardui* and *H. litura* is great in the western U.S. and state and federal agencies continue to invest considereable resources on rearing and redistrobution programs for both insects (M. Schwarzländer pers. comm.).

SITE SELECTION

The Idaho Biocontrol Task Force, a partnership between the University of Idaho, Idaho State Department of Agriculture, Nez Perce Tribe Biocontrol Center, USDA Forest Service, and USDI Bureau of Land Management, initiated in collaboration with the USDI

Bureau of Indian Affairs, an effort in 2008 to monitor changes of CT infestations and the surrounding plant community following releases of *U. cardui* and *H. litura* at permanent study locations, focusing on the states of Idaho and South Dakota. *Cirsium arvense* infestations for permanent monitoring were chosen following a request for study sites to county weed personnel and tribal land managers. The protocol used for monitoring was developed with the goal to allow and encourage land managers, stakeholders, and other citizen scientists to participate in the data collection process (ISDA, 2014). Most study sites were selected on the basis of accessibility and availability of local collaborators to participate in the data collection process. The potential bias of the study site selection process was weighed against logistical constraints of sampling a large number of sites over a wide geographic range within a short time period, and at locales often distant from transportation corridors, situated in rugged terrain or inaccessible due to ownership. The request led to the establishment of a total of 87 permanent study transects located throughout Idaho (n = 44), Utah (n = 8), Wyoming (n = 4), North Dakota (n = 5), and South Dakota (n = 26) (Table 3.1, Fig. 3.1). *Cirsium arvense* infestation size estimated in hectares, ecoregion type (level III, (EPA, 2013)), and habitat type were determined for each study transect (Ricketts, 1999). Study transects spanned 12 ecoregions, five degrees of latitude (41 to 46 ˚N), and habitat types ranging from temperate coniferous forests to xeric deserts. *Cirsium arvense* infestation patch size ranged from 0.01 to 2.2 ha. Study transect elevation ranged from 368 m (Site 40, Sisseton) to 2504 m (Site 28, Alpine) above sea level (Table 3.1).

Since the main objective of this study was to measure changes in vegetation following biological control insect releases, insects were released during the spring before transects were monitored for the first time or, alternatively, some study sites had already received fly or

weevil releases (since 2006). In addition, *C. arvense* biological control agents may have been released at study sites as part of earlier redistribution programs in the 1970s and 1980s but we had no records for this for any of the study sites. Each study transect received a release of either *U. cardui* adults, *H. litura* adults, both biological control insect species, or no insect releases (i.e., control site). For those study sites with known release records, between 50 to 400 insects (118 ± 10 insects, mean ± SE) were released between 2006 and 2009. Study transects were at least 1 km distant from each other. Because insects may have dispersed to other study transects over time or may have been present at study transects at the time of establishment, the abundance of both insect species was assessed at all study transects for each year. A study transect in which no biological control insect species was recorded despite its release was considered a control transect and transects at which either *U. cardui* or *H. litura* was recorded during any study year was re-categorized into the respective insect species release treatment. A number of study transects (n = 7) in the following treatments: *H. litura* released (n = 1), both insects released (n = 3), and control (n = 3) were removed from analysis due to repeated disturbance in the form of ungulate grazing, long-period flooding, or human construction. While pairing non-release control sites with respective release sites would have been the most appropriate approach to test biological control insect impact, this was not possible due to the widespread distribution of both insect species and unknown redistribution program history in combination with their cryptic biology. Nonetheless, this study may be useful to identify factors affecting CT population dynamics due to variation in time since *U. cardui* and *H. litura* releases, and variation of other biotic and abiotic study site variables.

INSECT MONITORING

Permanent 20 m transects were established near the center of CT infestations with a minimum size of 0.5 ha. Biological control insect abundance was assessed for *U. cardui* through repeated (n = 6) three-minute counts of galls (Eber and Brandl, 1994) in separate visually defined areas surrounding the transect, and indirectly estimated for *H. litura* as the proportion of stems mined through dissection of at least 30 randomly collected CT stems in the vicinity of the transect for each study site and each year. All transects were sampled in late summer after *U. cardui* gall formation was almost complete and *H. litura* larval galleries were clearly visible and mature larvae still found in stems.

EFFECT OF *U. CARDUI* AND *H. LITURA* ON INDIVIDUAL *C. ARVENSE* RAMETS

To assess the effects of *U. cardui* galling and *H. litura* mining on individual CT ramet performance, the stem height (cm), number of activated secondary stem buds, stem-base diameter (SBD) (mm), number of seed-heads produced, and dry weight (g) following drying for 24 h at 70 °C were measured for groups of ramets with *H. litura* larval feeding, *U. cardui* galls, or no herbivory at a subsample of study sites. Because of the low abundances of *U. cardui* and *H. litura*, this experiment was conducted at a subsample of sites in 2012 that were characterized by a relatively large abundance of one or the other insect species (i.e., Site 1, Nez Perce; Site 7, Bruneau; Site 20, Burley; Site 22, Bannock; Site 23, Great Feeder; Site 27, Soda Springs; Site 31, Logan; Site 32, Ogden). At each study site (n = 8), ramets attacked by insects (n = 30) and corresponding unattacked ramets (n = 30) were randomly collected in the proximity of the study transect and transported to the lab for analysis. We counted seed-heads in place of seeds because the number of seeds per seed-head has previously been shown

unaffected by insect herbivory, including that of *H. litura* (Larson et al., 2005). For *U. cardui*, the number of galls on the main stem and lateral branches were recorded. For *H. litura*, stems were dissected and all larval instars were counted. The proportion of the stem length mined by *H. litura* larvae (%) was also recorded for each attacked stem. In some cases, especially at study sites in South Dakota, stem mining was not caused by *H. litura* but by other stem-boring beetles, e.g. *Languria mozardi* (Latr.) (Coleoptera: Erotylidae: Languriinae) and consequently these stems were excluded from analyses.

In addition, CT ramets were individually labeled in spring 2012 at the same eight study transects, and the SBD and stem height were recorded for each ramet (n = 953) to compare performance changes between *H. litura* mined and unmined stems. The ramets were re-measured in early August 2012, then harvested and transported to the lab for dissection and analysis. Ramets were dried (24 h at 70 °C) and the above-ground biomass was weighed (g).

VEGETATION COMMUNITY MONITORING

Ten 0.125 m² (25 by 50 cm) quadrats were placed in 2 m intervals along each transect (n = 87) and vegetation community composition was characterized in six broad classes by estimating quadrat cover to the nearest percent in each quadrat. These classes were: Canada thistle (CA), other invasive broadleaf weeds and annual grasses (OW), forbs (FB), perennial grasses (GR), bare ground (BG), and litter (LT). *Cirsium arvense* stem density ($Stems_t$) and height of the tallest ramet ($Height_t$) in cm were recorded for each quadrat, study site and year. Between 2008 and 2012, all transects were monitored between 2 June and 25 September with an average monitoring date of 15 July. The monitoring date varied depending on the elevation and climate at study sites.

ABIOTIC ENVIRONMENTAL DATA

Abiotic environmental data were collected for all study sites to separate biological control effects from other potential confounding environmental factors. These were minimum, maximum, and mean annual temperatures (°C) and total annual precipitation (mm) for each study site from 2007 to 2012 (NOAA, 2012), gathered from the respective closest weather station (22.54 ± 4.95 km, mean ± SE distance to study site). Mean annual temperature ranged from 2.80 ± 0.47 °C in 2009 (Site 24, Teton River) to 13.00 ± 0.16 °C in 2012 (Site 7, Bruneau). Total annual precipitation ranged from 139.7 mm in 2008 (Site 13, Hot Springs) to 1138.4 mm in 2010 (Site 9, Moutnay). Elevation was included using geospatial raster aspect-slope maps, which were overlaid on study sites, in ArcGIS 10.0 (USDA, 2013), to record elevation, aspect, and slope (Lakes, 2009). Aspect was reclassified in ArcGIS into one of nine direction categories: four cardinal, four ordinal, and flat. Soil type, soil texture, and subsoil pH data was obtained from USDA–Natural Resources Conservation Service web soil survey data (Beaudette and O'Geen, 2009). Study transects were categorized into 1 of 12 soil texture classes based on the USDA textural triangle classification (Twarakavi et al., 2010). Study transect distance to surface water (m) was also added as abiotic variable to explain growth patterns at study sites with very low annual precipitation.

STATISTICAL ANALYSIS

The SBD was used as an independent variable to assess biological control insect effects on CT ramet performance because of its correlation with ramet stem height ($r^2 = 0.53$, $n = 330$), number of seed-heads produced per ramet ($r^2 = 0.52$, $n = 310$), and above-ground biomass ($r^2 = 0.71$, $n = 247$). Linear regressions with SBD as predicting variable were used to

estimate the seed-head production of ungalled stems (control) (n = 222), stems with a single *U. cardui* gall (n = 260), stems with multiple *U. cardui* galls (n = 346), or stems containing galls of varying diameter (mm). T-tests, ANOVAs, and Tukey's pairwise comparisons were used to compare growth parameters of attacked and unattacked ramets for both *U. cardui* and *H. litura.*

Data analysis of vegetation community cover and biotic and abiotic parameters affecting inter-annual CT stem density changes followed that described in Weed and Schwarzländer (2014) with a few exceptions: because here we included a larger number of biotic and abiotic variables, only those study transects with three or more years of complete data were included in the model selection process. Generally, study site rather than quadrat level data was used because herbivore abundance was only assessed on the study site level and the main objective of the analysis was to assess whether changes in CT stem density over time were associated with insect abundance. A discrete model of population dynamics (Royama, 1992; Turchin and Taylor, 1992) was used as a baseline for developing and comparing competing models to understand which factors best explain changes of CT ramet densities between years. The stem density rate of change (R_t) was derived from the formula, $R_t = \ln(Stems_{ti}) - \ln(Stems_{ti-1})$, where $Stems_{ti}$ was the mean CT stem density (0.125 m^{-2} $site^{-1}$ yr^{-1}) in year t at site i and $Stems_{ti-1}$ was the previous years' stem density at site i. Per capita population growth rates based on stem density were explored in order to test for direct density dependence, often seen in intraspecific competition (Bonsall et al., 2003; Buckley et al., 2001; Freckleton and Watkinson, 2002).

Study transect data were summarized by calculating means for CT stem density, vegetation cover proportions, and biological control agent abundance measures across

quadrats for each study year. The local vegetation community compositions, and associations within, were assessed through Pearson correlations of relative vegetation cover category (Table 3.2). A negative response (density dependence) was predicted on stem density in the present (*t*) or previous (*t-1*) year represented by the model; $R_t = Stems_t + Stems_{ti-1} + \varepsilon_t$. In this model, ε signifies sampling error in stem density counts and exogenous effects on *C. arvense* population dynamics (Berryman, 2003). An information theoretic approach was used to evaluate alternative competing models involving endogenous effects ($Stems_t$, $Stems_{ti-1}$, *Height*) and exogenous variables (Burnham and Anderson, 2002). Both current (*t*) and lagged years (*t*-1, *t*-2) biological control agent herbivory (*No. of galls* and *Transect mining*), vegetation cover categories (*Other weeds, Forbs, Perennial grasses, Bare ground, Litter*), total annual precipitation (*Precip*), distance to water (*Water*), time since biological control agent release (*Time*), site slope (*Slope*), site aspect (*Aspect*), subsoil pH (*Ph*), elevation (*Elev*), and mean minimum (*Min temp*), maximum (*Max temp*), and mean annual temperatures (*Temperature*) were assessed for influence levels as exogenous factors (ε) on changes in CT stem density during the study.

In order to test whether variables of the previous years (*t*-1, *t*-2) affected the current year (*t*) stem density, both current year and previous year's measures of annually fluctuating variables were included during the model selection procedures (Leathwick and Bourdot, 2012). Each variable was correlated with R_t and ranked by absolute correlative values; variables with little influence were removed. The three most correlative variables, along with biological control agent abundance variables, were used in the model selection procedure. This information theoretic approach compared the fit of all the possible candidate models nested within the following global model:

$Rt = Stems_{t-1} + Perennial\ grasses_t + No.\ of\ galls_{t-2} + Transect\ mining_{t-2} + Temperature$ + their two-way interactions

In order to explain changes in *Rt*, the main response variable, a multiple regression based model (Singer, 1998) was generated using SAS, Version 9.2 (SAS, 2014). Linear mixed effects models were used to estimate model parameters. Pearson rank correlations were used to test for association between R_t and possible model variables. No potential model variables were strongly associated, which allowed potential model inclusions of nearly all recorded variables.

Multi-model inference analyses, using second-order Akaike's information criterion (AICc), were conducted to test for the best fitting model, denoted by the lowest AICc score. Then models were ranked according to their difference in AICc score compared to the best fitting model. All models were considered plausible if the ΔAICc score was less than two. The model weight (w_i = relative likelihood of a model [$\exp(-0.5*\Delta AICc_i)$] divided by the sum of likelihoods of all models) was also calculated as confirmation of a model rank within those deemed most plausible. The relative strength of each predictor variable was ranked by summing the w_i values (Σw_i) across all of the models in which the predictor variable was present (Burnham and Anderson, 2002). After all plausible models were identified and ranked, the top ranking model was rerun as a mixed effects model with study site as a random classifier to calculate a new AIC value and significance level of each variable within the model.

Results

BIOLOGICAL CONTROL AGENT ESTABLISHMENT AND ABUNDANCE

Urophora cardui established at 68.6 ± 8.0% (mean ± SE, $n = 35$) of the study sites at which it was released (Fig. 3.2). Following the release of *U. cardui*, it took typically two years before the gall fly could be detected. In the years following the release of *U. cardui*, gall abundance did not change at study sites ($P = 0.21$, Fig. 3.3A). *Urophora cardui* gall abundance varied greatly among study sites and years and the gall flies were found widespread regardless of whether insects were released at study transects or not ($P = 0.125$, $t = 1.55$, $df = 65$); i.e., gall flies established at 50.00 ± 8.98% ($n = 32$) of previously un-colonized transects. The overall mean number of galls found during three-minute counts at *U. cardui* release sites was 2.59 ± 0.58 ($n = 98$). The overall mean proportion of CT ramets galled at *U. cardui* release sites was 3.46 ± 0.81% ($n = 129$) or 2.06% ($n = 4621$) of all stems surveyed at respective *U. cardui* sites.

The establishment rate for *H. litura* at study transects following weevil releases was 82.9 ± 6.5% (mean ± SE, $n = 35$) (Fig. 3.2). Although weevils established readily at release sites, populations did not increase over the course of our study ($P = 0.43$, Fig. 3.3B). The proportion of ramets mined by *H. litura* varied significantly between study sites and years ($P = 0.0015$, $F = 3.689$, $n = 89$), but generally, *H. litura* mines were found ubiquitously across study sites with 45.40 ± 3.20% ($n = 93$) of CT ramets mined at *H. litura* release sites. For the subset of study sites with higher *H. litura* abundances, at which stems were tagged in June 2012, 86.49 ± 1.99% ($n = 296$) of tagged ramets were mined though that value decreased in August to 64.04 ± 3.61% ($n = 178$, $P < 0.001$, $t = 5.91$, $df = 472$). At this subset of study sites, attacked stems contained in June 2012 4.24 ± 0.36 *H. litura* larvae (range 1 to 37).

EFFECT OF *U. CARDUI* AND *H. LITURA* ON INDIVIDUAL *C. ARVENSE* RAMETS

Ramets galled by *U. cardui* did not differ in individual ramet biomass, apical stem height, number of seed-heads produced, and SBD from ramets that were not galled (P = 0.319, t = 1.09, n = 425) (Fig. 3.4A). There was a trend towards reduced seed-head production in *U. cardui* galled ramets with increasing ramet size but the difference was only significant in one out of eight ramet size classes (i.e., SBD 7-8 mm, $P < 0.01$, t = 2.72, n = 47) (Fig. 3.5A). Stems with multiple *U. cardui* galls produced fewer seed-heads compared to stems with a single *U. cardui* gall or unattacked stems in the same ramet size classes ($P < 0.05$, q = 3.842, n = 35) but that effect disappeared in the largest stem size class (SBD > 8.0 mm, P = 0.442, F = 0.838, df = 67). Increasing *U. cardui* gall size (diameter in mm) did not negatively affect apical stem height of attacked ramets (P = 0.48, n = 22) or the number of seed-heads produced (P = 0.49, n = 22).

Across all study sites and years, *H. litura* larvae mined 28.26 ± 1.80% (mean ± SE, n = 87) of the lengths of CT ramets regardless of ramet size and that value did not differ between study sites (P = 0.86, F = 0.488, n = 61). *Cirsium arvense* ramets mined by *H. litura* (n = 203) weighed 152.64 ± 44.26% more ($P < 0.0001$, t = 5.86, df = 7), were 20.97 ± 29.24% taller (P = 0.001, t = 5.40, df = 7), had a 49.08 ± 8.33% wider stem-base diameter (SBD) (P = 0.0005, t = 6.11, df = 7), and produced 492.38 ± 174.04% more activated secondary stem growth (P = 0.024, t = 2.87, df = 7) than unattacked ramets (n = 712) (Fig. 3.4B). Across the entire study CT stem height did not differ between *H. litura* release sites and control transects without weevil releases (P = 0.262, F = 1.27, df = 185); however, stem height variation differed among insect release sites because *H. litura* attack occasionally led to greatly reduced stem height or even ramet death ($P < 0.0001$, Bartlett = 35.26). There was no difference in the

correlation between the number of seed-heads produced per ramet and the ramet size (measured in SBD) between *H. litura* mined and unattacked stems ($P = 0.93$, $F = 0.0073$, $df = 435$) but, ramets with > 60% of the stem mined by *H. litura* produced 50.91 ± 21.09% (mean ± SE) fewer seed-heads compared to ramets with < 20% of the stem length mined ($P = 0.018$, $t = 2.42$, $df = 93$) (Fig. 3.5B).

Of the 953 ramets tagged in June 2012 to study the effect of *H. litura* mining on ramet performance, 3.6% ($n = 35$) of apical stems broke off, 5.5% ($n = 53$) of stems were missing, and 16.1% ($n = 154$) of ramets were dead in August, respectively. At both census dates, ramets mined by *H. litura* had a larger stem-base diameter (June: $P = 0.0024$, $t = 3.04$, $df = 898$; August: $P < 0.0001$, $t = 11.02$, $df = 726$) and weighed more (June: $P = 0.0005$, $t = 3.54$, $df = 414$; August: $P < 0.0001$, $t = 5.86$, $df = 712$) than unattacked stems. Mined ramets were also taller than healthy ramets in June ($P < 0.0001$, $t = 11.02$, $df = 726$), but that difference disappeared in August ($P = 0.881$, $t = 0.15$, $df = 776$). When comparing *H. litura* mined ramets and unattacked ramets of equal stem height, attacked ramets produced 4.73 ± 2.28 (mean ± SE) more seed-heads than unattacked ramets ($n = 172$, $P = 0.034$, $F = 4.57$, $df = 169$).

The number of seed-heads produced per stem pooled across all study sites and years for *C. arvense* ramets that galled by *U. cardui*, mined by *H. litura*, attacked by both insects, or that were not attacked differed ($n = 1047$, $P = 0.01$, $F = 3.80$, $df = 3$). Ramets attacked by both insects produced fewer seed-heads compared to ramets that were attacked by either *U. cardui* or *H. litura* alone or to unattacked ramets ($P = 0.046$, $t = 1.99$, $df = 474$).

VEGETATIVE COMMUNITY CHANGES

Across all study sites and years, the vegetation cover in our broad measured categories in quadrats was as follows: *C. arvense,* 18.55 ± 0.38% (mean ± SE); other weeds, 4.43 ± 0.21%; forbs, 14.12 ± 0.37%; perennial grasses, 32.67 ± 0.52%; bare ground, 6.40 ± 0.27%; and litter, 23.83 ± 0.40%. The inter-annual rate of vegetation cover change for *C. arvense* ($P = 0.529$, $F = 0.742$, $df = 3$), or any other vegetation cover category ($P = 0.93$, $F = 0.15$, $df = 3$) were not different among insect release treatments (Fig. 3.6). However, across all study sites, these cover categories differed in inter-annual rate of change ($P = 0.002$, $F = 4.12$, $df = 191$) due to the increase in litter cover compared to the decrease in CT cover across the study. *Cirsium arvense* ramet density decreased during the study period ($P = 0.003$, $F = 4.10$, $df = 4$), but there was no difference in the annual rate of decrease between insect release treatments ($P = 0.62$, $F = 0.59$, $df = 3$, Fig. 3.6). There were significant negative correlation between *C. arvense* cover and bare ground, perennial grasses, and forb cover (Table 3.2).

FACTORS AFFECTING INTER-ANNUAL *C. ARVENSE* DENSITY CHANGE

Models considered plausible by our selection process ($\Delta AICc < 2$) included endogenous and exogenous factors (Table 3.3), inferring that both factor categories influence inter-annual changes in CT stem density. *Cirsium arvense* stem density in the previous year (t-1) was the most explanatory factor for *C. arvense* ramet density change in the model (Cuddington, 2011) and indicates direct density dependence (Table 3.4) but there was no correlation between stem density and stem density change rate (Fig. 3.7B). After removing the density dependent effect, perennial grass cover explained changes in CT stem density (Fig. 3.7A) and was the only vegetation cover category included in all plausible models

(Table 3.4). Despite the inclusion of mean temperature in all four models as a plausible factor, it was not a significant factor in the final global model (Table 3.4). Previous years (*t*-2) *U. cardui* gall abundance was not included in the top ranked model (Table 3.3) and failed to explain inter-annual changes in CT stem density (Table 3.3, Fig. 3.7C). *Hadroplontus litura* mining was included in the final model (Table 3.3) but failed to further explain changes in CT stem density (Table 3.4, Fig. 3.7D).

Discussion

BIOLOGICAL CONTROL AGENT ESTABLISHMENT AND ABUNDANCE

Urophora cardui did not establish uniformly well, with half of the releases failing to establish at *C. arvense* infestations in Idaho. In addition, fly populations rarely grew and remained low, only attacking a small proportion of available host plant ramets. These results were disappointing, although not unexpected given the previously reported low establishment rates and abundances in New Zealand (Gourlay, 2004) and the low host plant use in the United States (Goeden, 1987; Headrick and Goeden, 1996) and also its European native range (Eber and Brandl, 1994; Zwölfer, 1994). *Urophora cardui* abundance varied greatly among study sites and years in our large scale study. These results are consistent with findings on patch occupation in the native range. In Europe, *U. cardui* occupied 50 to 70% of host-plant patches, subpopulation mortality rates reached up to 100%, and annual within patch extinction and colonization rates reached up to 30% (Eber and Brandl, 1994; Schlumprecht, 1989). Establishment and abundance of *U. cardui* in our study were comparable to data found in earlier assessments of the fly in the United States (Liu et al., 2000; Reed et al., 2006) and comparable to values found for the gall fly in its native European range (Eber and Brandl,

1996). However, in order to inflict measurable impact on *C. arvense* populations, the fly would have to reach larger or outbreak densities in the introduced range (Myers et al., 1987), as is common for other successful classical biological control agents such as *Galerucella calmariensis* on Purple loosestrife (Landis et al., 2003) or *Mecinus janthinus* on Dalmatian toadflax (Van Hezewijk et al., 2010).

Similar to *U. cardui*, *H. litura* were found widespread in our study but in contrast to the gall fly, the weevil established consistently readily at release sites during our study. As for *U. cardui*, overall less than half of ramets available were mined by *H. litura* and those attacked contained typically few larvae. Our findings are matching those of other studies conducted in the states of North Dakota (Prischmann-Voldseth et al., 2012) and South Dakota (Reed et al., 2006), which found high establishment rates and low stem mining frequencies for the weevil. Given the large spatial scale of our study, the consistent low abundances of *H. litura* despite high establishment rates and the lack of any observable population increase at release sites during a four year period, the weevil does not seem to provide any biological control potential.

EFFECT OF *U. CARDUI* AND *H. LITURA* ON INDIVIDUAL *C. ARVENSE* RAMETS

Urophora cardui only had a measurable effect on seed-head reduction when multiple galls were found on a stem, or when ramets were also attacked by *H. litura*, both events that were rare given the low abundance of either insect at *C. arvense* infestations throughout our study. As for the establishment and abundance data, the lack of measurable effects of *U. cardui* on ramet performance parameters is corresponding with results of previous studies in which flies failed to build up sufficient herbivory pressure to affect stem length, flowering, or

seed-head numbers (Gourlay, 2004; Reed et al., 2006). The control of clonal invasive plants with insect herbivores that from galls is considered especially difficult because the plant may be able to compensate for herbivory through relocation of resources to unaffected ramets and thus would require particularly high gall attack (Hutchings and Wijesinghe, 1997). However, as our results and those of other studies have shown, high *U. cardui* gall attack does not occur on a large spatial level in either the native or any of the introduced ranges (Eber and Brandl, 1994; Goeden, 1987; Harris, 1997; Schlumprecht, 1989), maybe because of the complex host selection behavior of *U. cardui* (Daniels, 2004) or other unknown factors.

We found that *H. litura* mining can reduce stem height and seed-head production but only if ramets are heavily mined. However, overall, our data show that *C. arvense* ramets are able to tolerate or even overcompensate for *H. litura* attack at the scale we conducted that study. Other recent studies have found that *H. litura* mining has, at least the potential to, negatively affect apical stem height or seed-head numbers (Burns et al., 2013), but the data presented here is supported by several previous studies suggesting that weak *H. litura* attack can stimulate stem growth (Peschken and Wilkinson, 1981; Reed et al., 2006; Zwölfer and Harris, 1966). Even with many larvae in a single stem, *H. litura* can be limited to a proportionally short section of the basal stem or root crown through formation of gall-like tissue (Zwölfer and Harris, 1966), resulting in a decrease of the relative length of the mine when the stem is elongating in the growing season as we found in this study.

Herbivory that does not cause meristem replacement through activation of secondary stem buds, may stimulate compensatory growth in other *C. arvense* stem parameters (e.g., stem biomass root growth) (Ang et al., 1994; Mitchell and Davis, 1996) or even lead to overcompensation via increased root storage product (Sciegienka et al., 2011).

In our study, stems mined by *H. litura* had much more activated secondary stem growth, although only a small proportion of stems broke off or died back by other means. Stem mining that results in stem breakage or death allows for new aerial stem production via stimulation of axillary buds near the base of the damaged stem or via ramet root buds (Anderson et al., 2010; Klimeš, 2007). These auxiliary buds are repressed by the dominance of a superior stem but when this dominance disappears the buds, along with stockpiled carbohydrate stashes, allow the plant to compensate the meristem loss quickly (Demers et al., 2006; Donald, 1990; Henskens et al., 1996; Mitchell and Abernethy, 1995). While most *C. arvense* stems grow before midsummer, stem replacement later in the season tends to balance deaths in the early cohorts, resulting in a relatively static shoot population (Bourdot et al., 1995). The stems replacing earlier damaged ramets generally escape herbivory by *H. litura* and *U. cardui*. Our study did not address the level of compensatory stem replacement due to herbivore attack but the reduction in the proportion mined stems between spring and summer for *H. litura* is in concordance with theoretical expectation of ramet replacement and further illustrates why both *U. cardui* and *H. litura* are unlikely to inflict long-term ramet declines in *C. arvense* (Henskens et al., 1996). In sum, our data show on a larger spatial scale what other authors have found earlier or elsewhere. *Hadroplontus litura* fails to increase to population densities that could be damaging, and as a consequence, the larval feeding on nonessential plant tissues causes little if any damage, for which *C. arvense* compensates or potentially overcompensates with secondary stem growth (Burns et al., 2013; Peschken and Wilkinson, 1981; Reed et al., 2006; Zwölfer and Harris, 1966).

VEGETATION COMMUNITY CHANGES AND FACTORS AFFECTING INTER-ANNUAL *C. ARVENSE* DENSITY CHANGE

The goal of classical biological control is to decrease an invasive plants' density below a threshold at which it no longer causes unacceptable ecological or economic damage (McFadyen, 1998). Consequently, biological control success is measured in a reduction of weed density or vegetation community changes at the population level (Syrett et al., 2000). There was no expectation to see ground cover changes in *C. arvense* or any other vegetation cover category given that we did not find any noteworthy impact of either *U. cardui* or *H. litura* on *C. arvense.* Thus, it is not surprising that vegetation cover behaved more or less static over the five year study period.

Both *C. arvense* cover and ramet density decreased during the course of the study. Theoretically, the non-random site selection process may have contributed to the observed pattern. Land managers tend to suggest selection of larger invasive plant infestations as study sites. On a metapopulation level, these populations are more likely to be at a peak level and consequently more likely to decline over time (Crawley, 1996). However, our top ranked model describing inter-annual *C. arvense* density changes identified the previous years' stem density as a factor indicating negative plant feedback in CT through intraspecific competition (Mamolos and Kalburtji, 2001) and similarly, perennial grass cover was a significant factor in the top model explaining CT stem density changes. Perennial grass cover was also the only factor that was negative correlated with *C. arvense* growth rate indicating the susceptibility of CT to interspecific competition. Grass competition has previously been shown to impact CT negatively (Ferrero-Serrano et al., 2008; Ferrero-Serrano et al., 2011) and native grasses competitive ability may even increase when competing with *C. arvense* over longer periods of time (Ferrero-Serrano et al., 2011). Grasses have stronger negative effects on CT than forbs

(Larson et al., 2013) and this may or may not have been the case in our study, but due to the small relative proportion of forb cover in quadrats, it was not a factor during the model selection process. The relatively weak effects found in our model selection process were surprising. In another study on the invasive plant *Linaria dalmatica* using a very similar monitoring protocol to that reported here, effects were much more pronounced despite the fact that approximately only half the number of study sites were included in the study (Weed and Schwarzländer, 2014). One reason explaining our inability to better identify factors associated with the inter-annual change of *C. arvense* stem densities may be the extreme variability of data, which in turn is in part due to extreme disturbance events at study sites (e.g., ungulate grazing for flooding for long periods of time).

In sum, our data show that *C. arvense* ramet densities growth rates are negatively affected by negative plant feedback in the form of intraspecific competition and interspecific competition with perennial grasses. Specialist insect herbivory and abiotic site variables included in the analysis had no additional effect on the density change rates. Our data supports findings of other studies on best *C. arvense* management practices that suggest plant competition using forbs during establishment (Burns et al., 2013; Gabruck et al., 2013) and grasses thereafter (Bicksler and Masiunas, 2009; Bicksler et al., 2012), may provide the best restoration potential for the invasive thistle. Our results also reaffirm findings in Europe and North America that interspecific competition, not specialist herbivory, can have negative effects on CT plant densities (Friedli and Bacher, 2001a; Larson et al., 2013; Tichich et al., 2006). Although *C. arvense* does not profit from enemy release (ERH) in its introduced ranges (Cripps et al., 2010), we conclude based on the data presented here and in concordance with other studies, that the two approved biological control agents in the United States are not

able to exact impact on *C. arvense* (Cripps et al., 2011). Consequently, resources for the biologically based management of CT should be redirected towards the development of other, more effective biological control agents or other management practices such as restoration or re-vegetation projects.

Field studies that allow the assessment of biological control agent and host plant populations fluctuation as a function of inter-annual changes in biological and environmental factors and their respective feedbacks are useful because they allow the studies to record net cumulative effects (Maines et al., 2013; Seastedt and Pysek, 2011). The study presented here did not only allow measuring cumulative effects, but the model selection procedure also allowed separating and weighing those factors most affecting the density growth rate of CT (Blumenthal et al., 2009; Weed and Schwarzländer, 2014). This is important because in our case, a simplistic field assessment of the consequences of biological control insect releases on CT stem densities after five years could have potentially and falsely attributed a weed density decline to biological control. Thus, we recommend the incorporation of simultaneously acting exogenous and endogenous factors which may have competing or synergistic effects to attribute net changes in weed densities correctly.

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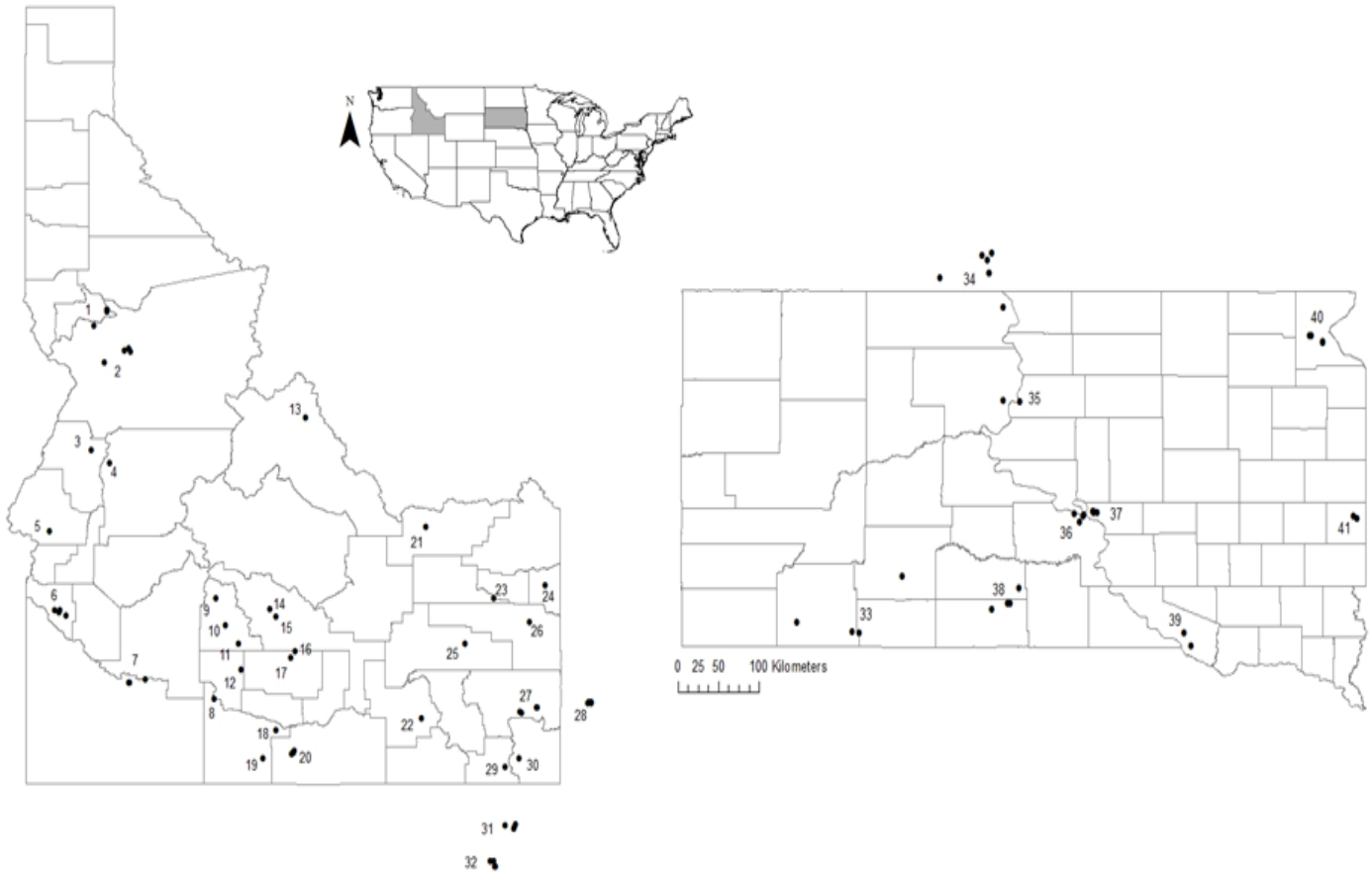

Figure 3.1 Study transect locations in the States of Idaho and South Dakota, and neighboring states (North Dakota, Utah, and Wyoming) in the north-central and northwestern USA (See also Table 3.1 for study site information).

Table 3.1 Populations of *Cirsium arvense* included in study.

No.	Site name	State	Transect[a]				Coordinates (NAD83)		Elevation (m)	Patch size (ha)	Land use	Ecoregion[b]	Habitat type
			F	W	B	C	Latitude	Longitude					
1	Nez Perce	ID	a	b	c	d	N 46° 30′ 35.4″	W 116° 33′ 48.8″	912	2.0	Rangeland	Columbia plateau	Temperate coniferous forests
2	Grangeville	ID	a	b	c	d	N 45° 47′ 18.0″	W 116° 09′ 20.0″	1475	0.2	Clearcut	Idaho Batholith	Temperate coniferous forests
3	Adams Co.	ID	a				N 45° 00′ 05.6″	W 116° 17′ 48.9″	1188	0.8	Recreation	Idaho Batholith	Temperate coniferous forests
4	McCall	ID	a				N 44° 52′ 58.8″	W 116° 05′ 25.7″	1555	0.4	Roadside	Idaho Batholith	Deserts and xeric shrublands
5	Washington Co.	ID	a				N 44° 16′ 10.0″	W 116° 45′ 49.1″	694	0.4	Roadside	Snake River Plain	Deserts and xeric shrublands
6	Nampa	ID	a	b	c	d	N 43° 32′ 03.8″	W 116° 39′ 32.0″	773	—	Lake shore	Snake River Plain	Deserts and xeric shrublands
7	Bruneau	ID	a	b	c	d	N 42° 53′ 45.5″	W 115° 50′ 37.4″	750	—	Lake shore	Snake River Plain	Deserts and xeric shrublands
8	Paradise	ID			c		N 42° 45′ 16.4″	W 114° 55′ 19.0″	889	0.2	Recreation	Snake River Plain	Deserts and xeric shrublands
9	Moutnay	ID			c		N 43° 40′ 10.2″	W 114° 53′ 55.1″	1751	0.4	Mt. drainage	Idaho Batholith	Temperate coniferous forests
10	Boy's Cabin	ID	a				N 43° 25′ 04.0″	W 114° 47′ 52.6″	1616	0.4	Alluvial plain	Idaho Batholith	Deserts and xeric shrublands
11	Spring Creek	ID	a				N 43° 15′ 29.1″	W 114° 39′ 09.7″	1559	2.0	Rangeland	Snake River Plain	Deserts and xeric shrublands
12	Toone's Thistle	ID			c		N 43° 01′ 21.1″	W 114° 37′ 26.8″	1149	0.4	Agricultural	Snake River Plain	Deserts and xeric shrublands

13	Hot Springs	ID	a			N 45° 17′ 47.3″	W 113° 53′ 43.1″	1155	2.0	Riparian	Middle Rockies	Temperate coniferous forests
14	Indian Creek	ID			c	N 43° 34′ 19.0″	W 114° 17′ 44.5″	1739	1.2	Lake shore	Idaho Batholith	Temperate coniferous forests
15	Spirit Woman N	ID			c	N 43° 29′ 52.7″	W 114° 13′ 36.0″	1681	0.4	Mt. drainage	Idaho Batholith	Temperate coniferous forests
16	Happy Bridge	ID	a			N 43° 11′ 29.8″	W 114° 01′ 08.7″	1416	0.4	Riparian	Snake River Plain	Deserts and xeric shrublands
17	Pagari Bridge	ID	a			N 43° 07′ 32.0″	W 114° 03′ 43.5″	1364	0.4	Riparian	Snake River Plain	Deserts and xeric shrublands
18	Murtaugh	ID			c	N 42° 28′ 31.2″	W 114° 13′ 40.5″	1312	2.3	Agricultural	Snake River Plain	Deserts and xeric shrublands
19	Shoshone Creek	ID			c	N 42° 13′ 08.8″	W 114° 22′ 33.8″	1804	2.2	Roadside	N. Basin and Range	Deserts and xeric shrublands
20	Burley	ID	a	b	c d	N 42° 16′ 13.9″	W 114° 02′ 29.9″	1516	0.1	Riparian	N. Basin and Range	Deserts and xeric shrublands
21	Medicine	ID			c	N 44° 18′ 47.9″	W 112° 33′ 16.8″	1804	0.4	Mt. riparian	Middle Rockies	Temperate coniferous forests
22	Bannock	ID			c	N 42° 34′ 42.3″	W 112° 36′ 07.7″	1539	2.0	Rangeland	N. Basin and Range	Deserts and xeric shrublands
23	Great Feeder	ID			c	N 43° 39′ 54.1″	W 111° 47′ 17.1″	1507	2.0	Roadside	Snake River Plain	Deserts and xeric shrublands
24	Teton River	ID			c	N 43° 46′ 56.4″	W 111° 12′ 52.9″	1819	2.0	Riparian	Snake River Plain	Deserts and xeric shrublands
25	Reid Place	ID			c	N 43° 15′ 27.8″	W 112° 06′ 58.6″	1404	2.0	Pasture	Snake River Plain	Deserts and xeric shrublands
26	South Snake	ID			c	N 43° 27′ 02.6″	W 111° 23′ 41.1″	1606	2.0	Roadside	Middle Rockies	Deserts and xeric shrublands

27	Soda Springs	ID	a	b	c	d	N 42° 40′ 56.6″	W 111° 18′ 09.6″	2001	0.1	Rangeland	Middle Rockies	Temperate coniferous forests
28	Alpine	WY	a	b	c	d	N 42° 43′ 26.5″	W 110° 44′ 19.2″	2504	—	Mountain valley	Wyoming Rocky Mts.	Temperate coniferous forests
29	Cub Canyon	ID			c		N 42° 08′ 30.7″	W 111° 39′ 53.7″	1694	0.4	Roadside	Wasatch Mountains	Temperate coniferous forests
30	Paris Canyon	ID			c		N 42° 13′ 22.2″	W 111° 30′ 41.5″	2153	0.8	Roadside	Wasatch Mountains	Temperate coniferous forests
31	Logan	UT	a	b	c	d	N 41° 37′ 20.7″	W 111° 33′ 03.3″	1821	<0.0	Roadside	Wasatch Mountains	Temperate coniferous forests
32	Ogden	UT	a	b	c	d	N 41° 17′ 45.6″	W 111° 49′ 35.8″	1498	0.3	Lake shore	Wasatch Mountains	Temperate coniferous forests
33	Pine Ridge	SD	a	b	c	d	N 43° 34′ 36.6″	W 101° 35′ 49.6″	804	0.1	Agricultural	High Plains	Temp. grasslands/shrublands
34	Standing Rock	ND	a	b	c	d	N 46° 02′ 45.0″	W 101° 10′ 43.7″	626	0.2	Roadside	NW Great Plains	Temp. grasslands/shrublands
35	Cheyenne River	SD	a				N 45° 01′ 32.1″	W 100° 17′ 36.6″	493	—	Riparian	NW Great Plains	Temp. grasslands/shrublands
36	Lower Brule	SD	a	b	c	d	N 44° 04′ 24.0″	W 099° 35′ 28.0″	467	0.1	Roadside	NW Great Plains	Temp. grasslands/shrublands
37	Crow Creek	SD	a	b	c	d	N 44° 06′ 44.0″	W 099° 28′ 44.0″	457	0.8	Rangeland	NW Great Plains	Temp. grasslands/shrublands
38	Rosebud	SD	a	b	c	d	N 43° 21′ 02.5″	W 100° 24′ 14.8″	711	<0.0	Rangeland	NW Great Plains	Temp. grasslands/shrublands
39	Yankton	SD		b	c		N 43° 00′ 13.1″	W 098° 23′ 18.5″	473	<0.0	Agricultural	NW Glaciated Plains	Temp. grasslands/shrublands
40	Sisseton	SD	a	b	c	d	N 45° 30′ 58.9″	W 096° 55′ 35.6″	368	0.8	Unused field	N. Glaciated Plains	Temp. grasslands/shrublands
41	Flandreau	SD		b	c		N 44° 03′ 42.0″	W 096° 33′ 05.4″	468	—	Riparian	W. corn belt plains	Temp. grasslands/shrublands

[a], Insect release treatments for study transects: F, *U. cardui*; W, *H. litura*; B = Both insect species released; and C, No insects released (Control); [b], Ecoregions classification (Level III) (EPA, 2013).

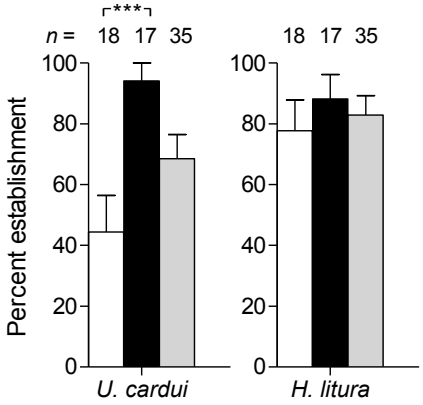

Figure 3.2 Proportion of sites at which *U. cardui* and *H. litura* established post-release for study transects in Idaho (white bars), South Dakota (black bars), and all sites combined (grey bars). *U. cardui* establishment rates different between Idaho and South Dakota study transects (***$P < 0.001$). Establishment rates were not different between *U. cardui* and *H. litura* ($P > 0.05$).

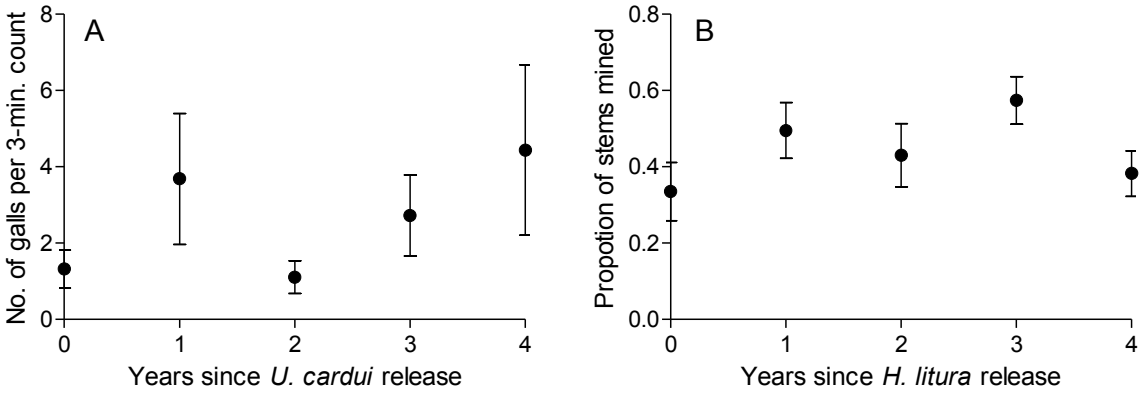

Figure 3.3 Insect abundance (means ± SE) of *U. cardui* (A) and *H. litura* (B) at release sites between 2008 and 2012.

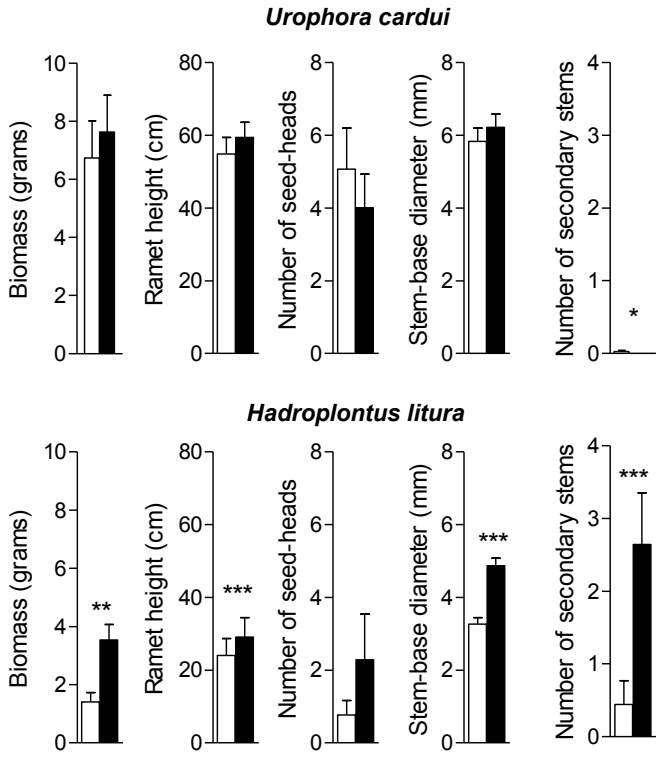

Figure 3.4 *Cirsium arvense* growth parameters for *U. cardui* galled and corresponding healthy stems (top row of graphs) and *H. litura* mined stems and corresponding healthy stems (bottom row of graphs). White bars represent unattacked stems and black bars are galled or mined stems. *$P < 0.05$, **$P < 0.01$, *** $P < 0.001$.

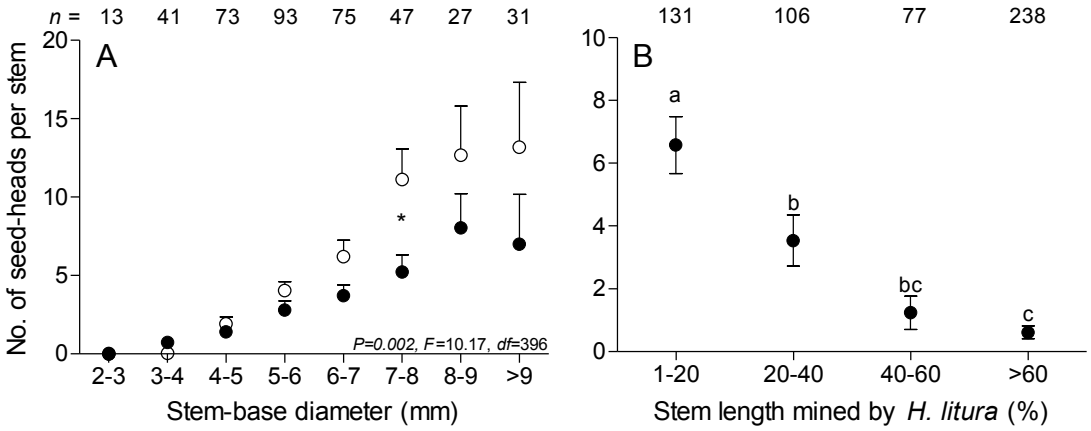

Figure 3.5 *Cirsium arvense* seed-head production (means ± SE) of *U. cardui* galled stems (black circles) in different stem-base diameter classes and corresponding unattacked stems (white circles, A) and *H. litura* mined stems in different mine length classes (B). **P* < 0.01, Bonferroni post-hoc multiple comparison. Seed-head production per ramet was different among different mine length classes (*P* < 0.0001, One-way ANOVA).

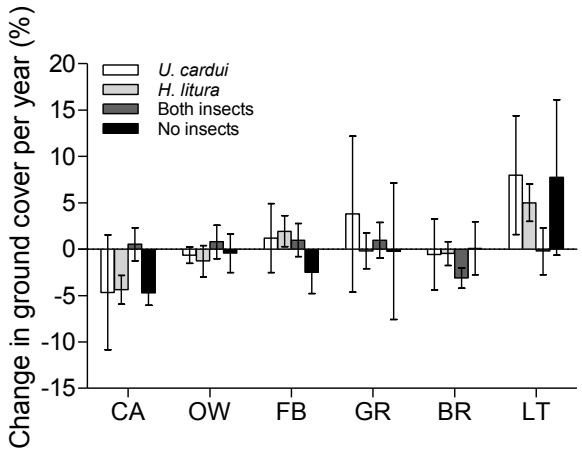

Figure 3.6 Yearly vegetation cover variation (mean ± SE) between 2008 and 2012 across all insect release study transects (n = 67) (CA, Canada thistle; OW, Other weeds; FB, Forbs; GR, Perennial grasses; BG, Bare ground; LT, Litter). *U. cardui* (n = 7), *H. litura* (n = 20), both insect species released (n = 33), no insects released (n = 7).

Table 3.2 Correlations among vegetation cover classes over the study period.

	Pearson correlation coefficients				
	Cirsium arvense	Bare ground	Perennial grasses	Other weeds	Forbs
Cirsium arvense					
Bare ground	-0.20***				
Perennial grasses	-0.44***	-0.20***			
Other weeds	0.01	-0.02	-0.22***		
Forbs	-0.24***	<0.01	-0.28***	-0.13*	
Litter	-0.01	-0.17**	-0.48***	-0.11*	-0.18***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 3.3 Plausible models (ΔAICc < 2) explaining inter-annual changes in stem density of *Cirsium arvense* between 2008 and 2012. Shown are regression coefficients estimated using maximum likelihood.

	Model rank	1	2	3	4
	ΔAICc	0.00	1.48	1.75	1.84
	Model weight (w_i)	0.44	0.21	0.18	0.17
$\sum w_i$					
-	Intercept	0.977	0.942	0.734	0.884
1.00	Perennial grass	-0.010	-0.009	-0.007	-0.010
1.00	Temperature	-0.075	-0.074	-0.077	-0.072
0.83	Transect mining$_{t-2}$	-0.004	-0.004	-0.004	
0.82	Stems$_{t-1}$	-0.044	-0.042		-0.046
0.21	No. of galls$_{t-2}$		0.031		

Table 3.4 Factors included in the best-fitting model ranked by the relative importance of each predictor in inter-annual fluctuations in *Cirsium arvense* stem density.

Factors*	P-value	F-value
Perennial grass	0.005	8.31
Temperature	0.442	0.60
Transect mining$_{t-2}$	0.443	0.60
Stems$_{t-1}$	0.003	9.47

*All variables $df = 63$.

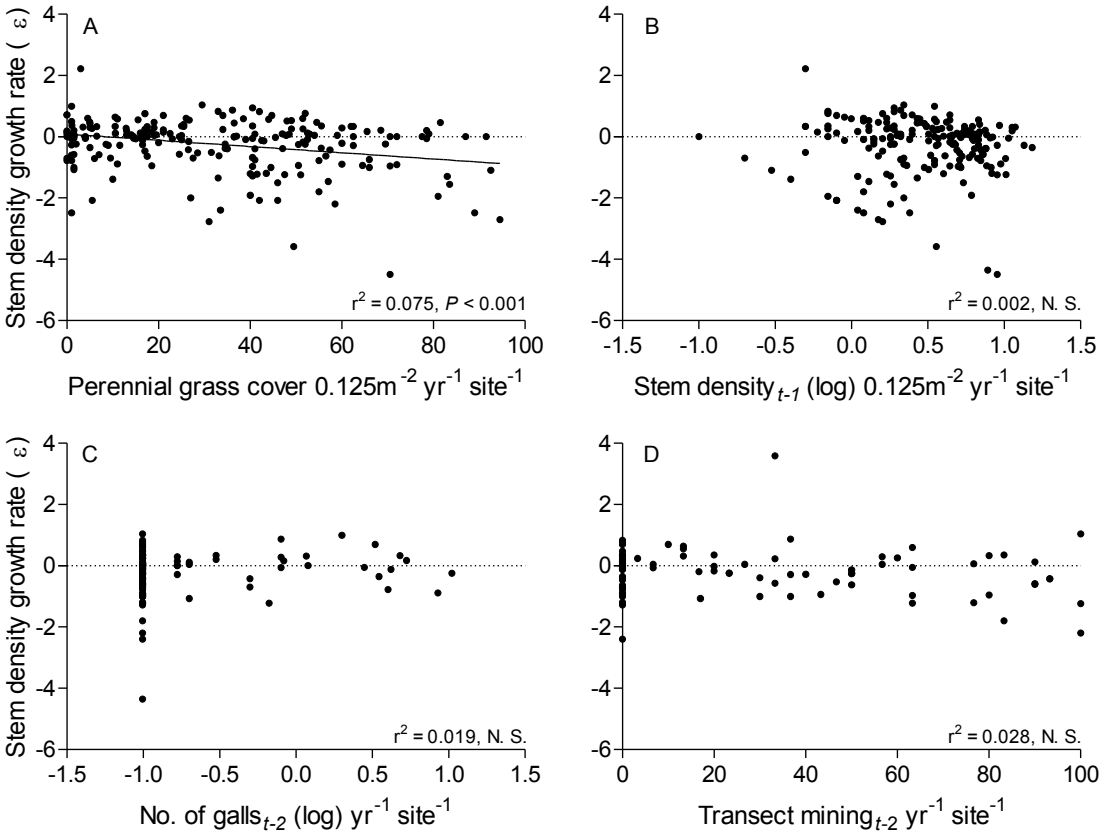

Figure 3.7 Relationship between (A) perennial grass cover, (B) previous year *C. arvense* stem density (C) previous year *U. cardui* gall abundance, or (D) previous year *H. litura* mine abundance and inter-annual changes in *Cirsium arvense* stem density (i.e., growth rates) between 2008 and 2012.

Appendix A

Arthropod species reared from *Urophora cardui* galls that composed less than two percent of total non-*U. cardui* emergence.

Appendix A Arthropod species reared from *Urophora cardui* galls that composed less than two percent of total non-*U. cardui* emergence.

Species	Order	Family	Guild	States present
Acarid sp.	Acariformes	Acaridae	Scavenger	ID, OR, MT, WA, SD
Tricorynus sp.	Coleoptera	Anobiidae	Inquiline	SD
Dolichosoma foveicolle (Kirby)	Coleoptera	Cleridae	Predator	OR, WA
Enoclerus rosmarus (Say)	Coleoptera	Cleridae	Predator	SD
Phyllobaenus humeralis (Say)	Coleoptera	Cleridae	Predator	ND
Isohydnocera curtipennis (Newman)	Coleoptera	Cleridae	Predator	MT
Mordellistena sp. A	Coleoptera	Mordellidae	Facultative[1]	SD
Mordellistena sp. B	Coleoptera	Mordellidae	Facultative	SD
Larinus planus (Fabricius)	Coleoptera	Curculionidae	Facultative	OR
Gasteruption sp.[a]	Hymenoptera	Gasteruptiidae	Inquiline	MT
Bracon sp.	Hymenoptera	Braconidae	Parasitoid	ID, OR, SD
Schizoprymnus sp.[b]	Hymenoptera	Braconidae	Parasitoid	ID, OR, SD
Pimplinae spp.	Hymenoptera	Ichneumonidae	Parasitoid	OR, MT
Mesopolobus sp.	Hymenoptera	Pteromalidae	Parasitoid	OR
Trichomalopsis dubia (Ashmead)	Hymenoptera	Pteromalidae	Parasitoid	OR
Lep. A	Lepidoptera	—	Facultative	MT
Lep. B	Lepidoptera	—	Facultative	ID
Sciarid sp.	Diptera	Sciaridae	Inquiline	OR, MT, WA, SD

[a], Predator of solitary bee and wasp larvae; likely entered gall to attack inquiline; [b], polyphagous insect known to attack *Mordellistena* spp. and other endophytophagous beetles; [c], secondary parasite of braconid, ichneumonid, and tachinid families. [1], Facultative insects listed are primarily phytophagous but opportunistically feed on *U. cardui* larvae.