FACTORS INVOLVED IN THE SUCCESS AND ESTABLISHMENT OF THE FIELD BINDWEED GALL MITE *ACERIA MALHERBAE* NUZZACI (ACARI: ERIOPHYIDAE)

by

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ABSTRACT

Despite years of study and management, field bindweed (*Convolvulus arvensis* L., Fam.: Convolvulaceae) remains a problematic invasive species across the United States and is in need of better management options. Studies were conducted to determine factors affecting the establishment and performance of the biological control agent *Aceria malherbae* Nuzzaci. To determine the effects of sub-lethal herbicide applications on gall induction and development of *A. malherbae*, a bioassay was conducted with four herbicides, each having different modes of action. Atrazine, glyphosate, imazapic, and picloram were applied at 25% of their recommended dosages on plants infested and not infested with *A. malherbae*. Sub-lethal herbicide applications had an adverse effect on plant stem height, total stem length, numbers of leaves or branches, or on above-ground or below-ground biomass; whereas *A. malherbae* did not. Synergistic impacts of herbicide applications and *A. malherbae* on growth parameters of field bindweed were not observed. Pre- and post-spray gall counts were not significantly different, indicating that gall induction and development was not altered by these sub-lethal dosages. The establishment and effectiveness of *A. malherbae* has been reported to vary across western North America, with genetic variation of field bindweed as a possible contributing factor. Four field bindweed populations, collected from Montana, California, Oregon, and New Mexico, were exposed to *A. malherbae* to determine if growth parameters conducive or detrimental to the development of the mite vary among plant populations. When grown in a common environment, plant height, stem length, and number of branches and leaves significantly varied among populations although biomass did not differ. Galling by *A. malherbae* did not impact field bindweed growth, except for slight reduction in root biomass of infested plants. Gall induction was lower on plants from New Mexico than Oregon. Field studies assessed the relationship between habitat characteristics and plant cover and the presence and abundance of the mite. Multidimensional scaling of site characteristics indicated a spatial relationship, though no habitat relationship, among established *A. malherbae* populations. In within-field studies, a significant positive relationship was observed between percent grass cover and mite abundance and a negative relationship between field bindweed and mite abundance.

CHAPTER 1

LITERATURE REVIEW

Background and Statement of Interest

Despite years of study and management efforts, field bindweed (*Convolvulus arvensis* L. (Fam.: Convolvulaceae)) remains a problematic invasive species across much of the United States and is in need of better management options, including that of biological control. In 1989 the field bindweed gall mite *Aceria malherbae* Nuzzaci (Acari: Eriophyidae) was first introduced into the United States (Bolt & Sobhian, 1993). Between 1992 and 1995 populations of the mite were introduced and established in Montana; however *A. malherbae* establishment and distribution remains patchy (McClay et al., 1999). Smith et al. (2010) suggested that late freezes, snow, or hail after mite emergence from overwintering could impact the mites' ability to develop populations that are damaging to field bindweed's biomass and reproduction. Smith et al. (2010) also noted that drier and more open sites have typically been associated with better *A. malherbae* establishment. However climate is not the only factor likely to affect the establishment of *A. malherbae*. Differences in management efforts, plant biotype, and habitat characteristics within sites can also be related to the success of *A. malherbae* and its impact on field bindweed. This thesis examined how various classes of herbicides affect *A. malherbae* gall induction, the relationship between regional field bindweed populations and successful gall induction, and habitat factors affecting establishment and performance of the mite in more northern climes. As a result of this research, a better understanding of the factors that affected post release *A. malherbae* effectiveness was considered.

Field Bindweed Distribution and Biology

Field bindweed is one of more than 84 common names associated with the problematic weed, *Convolvulus arvensis* L. (Fam.: Convolvulaceae). This perennial plant was brought to the United States in contaminated crop seed in 1739 from Eurasia (Boldt & Sobhian, 1993; Mitich, 1991; Weaver & Riley, 1982). It is also likely that it was planted as an ornamental or for medicinal purposes (Mitich, 1991). The weed has since spread rapidly throughout the United States, causing economic loss. It is present in the 48 contiguous states and Hawaii, and has been listed on the noxious weed list in 22 states (USDA NRCS, 2013). Field bindweed is commonly found in cultivated fields, pastures, gardens, lawns, waste areas, roadsides, and railways (Weaver & Riley, 1982). In the Great Plains, field bindweed infestations commonly cause yield reduction in cereal crops by 20-50 % (Boldt et al., 1998; Peterson & Stahlman, 1989). This invasive species is also a problematic weed throughout the world. It is distributed from 60°N to 45°S, and it is present in 44 countries and problematic in 32 different crops, resulting in its being among the world's worst weeds (Holm, 1977; Jacobs, 2007; Mitich, 1991; Weaver & Riley, 1982).

Field bindweed has viney growth habits and long-lived seeds. The vines can cause problems by being entangled in farm equipment during harvest. The vines also enable bindweed to climb onto crop plants or form dense mats of vegetation where there is no structure for the vines to climb. Since the seeds are small and ripen at the same time as many cereal crops, they are easily harvested along with the grain (Hanson et al., 1943). Hanson et al. (1943) reported over 36,000 field bindweed seed per 36.4 liters (one bushel) of harvested small grain crops in Nebraska. The amount of seed produced can vary considerably among locations and years due to both plant genetics and weather (Sherwood & Fuelleman, 1948). Seed production increases during drier years (Hanson et al., 1943), consequently field bindweed is a particular problem in the arid West. Field bindweed is also an obligate outcrosser (Sherwood & Fuelleman, 1948) and is pollinated primarily by insects. Seeds are not only carried from field to field and longer distances by harvesting and highway equipment, but can also be transported by passing through the digestive system of grain fed and grazing animals still viable (Hanson et al., 1943). The seeds may remain viable under field conditions for greater than 20 years (Timmons, 1949) with stored seeds maintaining 62% viability after 50 years (Brown & Porter, 1942). Because the seeds remain viable for decades and can easily spread, the seed producing capabilities of field bindweed make management difficult.

Field bindweed plants can also reproduce vegetatively via an extensive root system consisting of lateral roots, deep taproots, and rhizomes. The roots can reach a depth of nine meters and can compete effectively for limited water, survive long drought periods, and effectively compete against crops for limited water (Sherwood & Fuelleman, 1948; Weaver & Riley, 1982). The plants can also spread five meters laterally in a single year (Weaver and Riley, 1982), quickly enabling small infestations to significantly expand (Sherwood & Fuelleman, 1948). Root growth is dependent on soil and water table conditions (Frazier, 1943b; Sherwood & Fuelleman, 1948). Field bindweed may also spread via fragmented rhizomes which can grow into new plants (Sherwood & Fuelleman, 1948). New plants may also be produced from rhizome buds as deep as 60 cm below the soil surface. Tilling and repetitive cutting can serve to deplete the underground carbohydrate reserves of the field bindweed roots. Because the plants have a deep taproot and can effectively spread by vegetative reproduction, managing field bindweed with these cultural methods is often only effective on small, newly formed infestations (Sherwood & Fuelleman, 1948).

Field bindweed biotypes, with distinct flowering and leaf characteristics, have been described (Brown, 1946; Degennaro & Weller, 1984). Such variation may affect management practices. The clonal reproduction combined with the seed produced by obligate outcrossing creates a highly genetically diverse population that can reproduce and spread quickly (Ward et al., 2008).

Management

Management options for field bindweed include mechanical, cultural, chemical, and biological control (Inderjit, 2009; Jacobs, 2007; Peachey, 2012; Wiese et al., 1997). Cultural control methods, such as competitive crops, plastic mulch, and repeated mowing, hoeing, or tilling, are particularly effective during the flowering stage because the carbohydrate reserves in the roots are reduced at this phenological stage (Hanson et al., 1943; Sherwood & Fuelleman, 1948; Wright, 2011). Bakke et al. (1939) found that while the root reserves of continuously tilled field bindweed were diminished closer to the soil surface, the roots below one meter contained sugars and nitrogen at levels higher than in the untreated control plots. Field bindweed plants can also photosynthesize normally even when under dense crop competition (20-30% light) and can become dormant or semi-dormant (Bakke et al., 1939), making competitive cropping a risky management option.

Herbicide use, like cultural and mechanical control methods, is effective after repeated use. Chemical control tactics also suffer some of the same problems as the mechanical and cultural methods. For example, repeated applications, often across multiple growing seasons and with different herbicide types, may be necessary to control field bindweed (Stone et al., 2005). The below ground reserves that remain after repeated mechanical and cultural management can also remain after herbicide application (Inderjit, 2009; Jacobs, 2007; Peachey, 2012; Wiese et al., 1997). Since herbicides are fairly expensive and may not be useable in some management settings, their utility can also be limited. Therefore, although cultural, mechanical, and chemical methods can be effective at managing or even eradicating field bindweed in individual fields, additional management methods are necessary to more completely manage field bindweed over large landscape scales.

Herbicide Effects on Plants

Herbicides are often chosen as a preferred management tactic since they are typically selective, rapidly kill plant growing points, and are less expensive than manual removal techniques such as hand weeding. However, problems such as resistance to continuously used herbicides of the same mode of action may develop (Mallory-Smith & Retzinger, 2003; Menne, 2005). Application or rotation of different classes of herbicides may help mitigate potential resistance. Herbicides are grouped by modes of action based on the effects the herbicide has on specific physiological or biochemical pathways (Mallory-Smith & Retzinger, 2003; Menne, 2005). Some of these groupings consist of several chemical families that vary in their chemical composition but impact plants equivalently and cause similar symptoms (Armstrong, 2012). Herbicide groups are based on globally recognized characterization by the Herbicide Resistance Action Committee (HRAC) and Weed Science Society of America (WSSA) (Mallory-Smith & Retzinger, 2003; Menne, 2005).

The earliest synthetic herbicides were developed in the 1940s. 2,4-Dichlorophenoxyacetic acid (2,4-D) was the first to be produced and has since become one of the most widely used herbicides (Burns et al., 2001; Mithila et al., 2011). Phenoxy herbicides such as 2,4-D and 2,4-DB (4-(2,4-dichlorophenoxy)butyric acid) affect the growth of the plant by mimicking auxins and attaching to the growth receptors to induce changes in cell division and stem elongation that often result in plant death. Other auxin mimics (HRAC/WSSA group 4(o)) in distinct chemical families have been developed since; these herbicides include: picloram, a picolinic acid derivative in the pyridine family, dicamba, a benzoic acid derivative, and quinclorac in the quinoline carboxylic acid family. Since it is likely that these compounds act on a variety of biochemical sites within the plant, few examples of resistance to auxinic herbicides have been detected (Mithila et al., 2011; Sterling & Namuth, 2004).

While the auxinic herbicides affect plant growth, amino acid inhibitors target amino acid production pathways in the plant. These include 5-enolpyruvylshikimate-3-phosphate synthase (EPSP synthase) inhibitor, branched-chain amino acid inhibitors (acetolactate synthase (ALS) inhibitors), and glutamine synthetase inhibitors, which inhibit the enzymes that incorporate inorganic nitrogen to produce an amino acid (Duke, 1990). The aromatic amino acid inhibitor acts to inhibit EPSP synthase, blocking the production of the aromatic amino acids phenylalanine, tyrosine, and tryptophan. ALS inhibitors act on plants by inhibiting branched-chain amino acid production, causing a deficiency in leucine, isoleucine, and valine (Armstrong, 2012; Duke, 1990). Within the ALS herbicides, distinct families composed of herbicides with distinct chemistries includes: imidazolinones, sulfonylureas, and sulfonanalines (Duke, 1990). Though multiple resistance to several ALS inhibitors can occur, the differences in the chemical structures in the ALS inhibitor family results in disparities in the binding sites of the herbicide. Therefore, plants resistant to one ALS group (such as sulfonylureas) might not be resistant to another ALS group (such as imidazolines).

Other commonly used herbicides act on photosynthesis. Photosynthesis II inhibitors, such as atrazine encompass numerous chemical families and account for the most common herbicide mode of action (Ross & Childs, 2012). These inhibitors bind to the D-1 site of the quinone binding protein and block electron transfer following light absorption during photosynthesis (Duke, 1990; Nag et al., 2011; Namuth Covert, 2012).

Photosystem I inhibitors, which include the bipyridyliums paraquat and diquat, also affect photosynthesis. These herbicides scavenge electrons produced during photosynthetic electron transfer and reduce oxygen resulting in superoxide radicals which in turn damage membranes and bleach pigments (Duke, 1990; Ross & Childs, 2012). Protoporphyrinogen oxidase (PPO) inhibitors cause an accumulation of chlorophyll precursors which react with oxygen to form free radicals which also cause photobleaching (Duke, 1990; Ross & Childs, 2012). Carotenoid pigment inhibitors cause white new growth with pink and purple tinges as they block terpenoid synthesis and reduced carotenoids eliminates their photo protection so chlorophyll degrades (Duke, 1990).

Cell division disruptors inhibit microtubule assembly (Mallory-Smith & Retzinger, 2003). Within this group are the phosphoric amide and dinitroaniline herbicides, which disrupt cell division by binding to tubulin, the protein from which microtubules, essential in cell division, are made (Duke, 1990). Other cell division disruptors affect microtubule assembly and microtubule associated proteins (Duke, 1990). Cellulose synthesis inhibitors such as dichlobenil alter cell division processes and inhibit shoot and root growth (Ross & Childs, 2012). Lipid biosynthesis inhibitors block acetyl-coa carboxylase (ACCase), involved in fatty acid production and cause discoloration and disintegration of grass nodes and rhizome nodes (Ross & Childs, 2012).

Herbicides Recommended for Use on Field Bindweed

Although many herbicides have been tested on field bindweed, only auxinic mimics and amino acid inhibitors are recommended for use (Peachey, 2012); but herbicides commonly need to be applied across several seasons to effectively control the plant. Often, in broadleaf crop situations, the use of herbicides to control field bindweed will also negatively affect crop success, prohibiting a given herbicide's use. Often it cannot be applied at levels adequate to diminish field bindweed encroachment without crop damage.

Of the multiple herbicides recommended for field bindweed management, 2,4-D is commonly used along roadsides and railroad rights-of-way. Similarly, glyphosate, imazapic, picloram, and atrazine are used to directly combat field bindweed or other weed species present within field bindweed infested areas. Atrazine has been reported to be effective on field bindweed (Agamalian et al., 2012; Jemison et al., 2007), although it is not labeled for this use.

Biological Control

While cultural, mechanical, and chemical management options for field bindweed are often cost effective for high value land or for small areas, biological control is often considered for weeds, such as field bindweed, that need to be managed on a landscape scale. Classical biological control refers to the formal process of identifying natural enemies of the target weed, testing to ensure that the proposed biological control agent is likely to be non-damaging in the introduced area, and then introducing the natural enemy into the introduced range to keep the biological control agent below an economic threshold level of the weed (McFadyen, 1998). When successful classical biological control is implemented, it has the benefit over other management methods in that the agent can reproduce and disperse to continuously impact populations of the target weed, typically over an area beyond what a single land manager can administer (McFadyen, 1998). The dispersal and reproduction capabilities also enable biological control agents to impact areas where other control methods are not an option, such as near sensitive water ways or difficult terrain.

The goal of biological control is often to keep the target pest below the defined economic threshold, rather than complete eradication. Classical biological control is long termed and is considered highly cost effective when successful (Chalak et al., 2011; Hill & Greathead, 2000; Nordblom et al., 2002). For some target weeds, biological control has already saved millions of dollars in continued management costs (McFadyen, 1998), but typically the benefits of a particular biological control agent on a target weed are less quantifiable.

History of Biological Control on Field Bindweed

In the 1970s the United States Department of Agriculture and the University of California investigated field bindweed's natural enemies (herbivores and pathogens) in several Mediterranean and Middle Eastern countries for possible use as biological control agents (Rosenthal et al., 1983). The selection of potential biological control agents for field bindweed was challenging due to the close phylogenetic relationship(s) between field bindweed and important crop species within the Convolvulaceae, including sweet potato, *Ipomoea batatus* (L.), as well as native *Calystegia* species present in the United States (two species of which, *C. piersonii* (Abrams) Brummitt and *C. stebbinsii* Brummitt were listed as threatened or endangered in California) (Rosenthal et al., 1983; Rosenthal & Platts, 1990). About 150-200 herbivore and pathogen species were found associated with field bindweed in its native range (Rosenthal et al., 1983; Rosenthal & Buckingham, 1982; Rosenthal & Platts, 1990), three of which underwent host specificity testing. These species were a chrysomelid beetle, *Galeruca rufa* (Germar) (Rosenthal & Hostettler, 1980), a defoliating moth, *Tyta luctuosa* (Denis & Schiffermuller) (Lepidoptera: Noctuidae) (Rosenthal et al., 1988), and the bindweed gall mite *Aceria malherbae* Nuzzaci (Acari: Eriophyidae) (Nuzzaci et al., 1985). *Aceria malherbae* was initially identified as *Aceria convolvuli* (Nalepa) but later classified as a distinct species (McClay et al., 1999; Nuzzaci et al., 1985). Two of these biological control agents, *T. luctuosa* and *A. malherbae* were approved for release for the management of field bindweed in the United States (Littlefield, 2004). Both *A. malherbae* and *T. luctuosa* can complete development on some *Calystegia* species related to field bindweed (Chessman et al., 1997; Rosenthal et al., 1988; Rosenthal & Platts, 1990; Tipping, 1997). A population of *T. luctuosa* has recently been found to be established in western Colorado (Colorado Department of Agriculture, 2013).

In 2009, CABI Switzerland restarted field bindweed biological control agent testing (Cortat, 2013). Two species of flea beetles, *Longitarsus pellucidus* Foudras and *Longitarsus rubiginosus* Foudras (Coleoptera: Chrysomelidae), were studied. Both had been previously identified by Rosenthal and Buckingham (1982) as potential biological control agents but had not been thoroughly tested for host specificity. While *L. rubignosus* has been eliminated as of 2013 from testing consideration because it lacks host specificity, *L. pellucidus* is still being evaluated (Cortat, 2013). CABI is also testing a stem boring fly, *Melanagromyza albocilia* Hendel (Diptera: Agromyzidae).

Melanagromyza albocilia can feed and lay eggs on native North American *Convolvulus* and *Calystegia* species, although at levels lower than those on field bindweed, indicating a preference for field bindweed (Cortat, 2013). Further tests of *M. albocilia* will be conducted by CABI to determine if larval development can occur on the native *Convolvulus* and *Calystegia* species (Cortat, 2013).

Aceria Malherbae Distribution and Damage

Aceria malherbae, is a microscopic eriophyid mite native to southern Europe including Italy, Greece, Spain and southern France (Rosenthal & Platts, 1990). After host specificity studies were complete, *A. malherbae* was permitted in 1987 by the USDA-APHIS for release in the United States (Boldt & Sobhian, 1993). Although it was initially targeted to be released in California, the presence of a closely related *Calystegia* species in California prohibited its release in the state (Boldt & Sobhian, 1993; Rosenthal & Platts, 1990). Instead, *A. malherbae* was released near Temple, Texas in 1989 (Boldt & Sobhian, 1993). Releases were also made in North Dakota, Oklahoma, and New Jersey (Boldt & Sobhian, 1993), Kansas (Nechols, 1996), and Canada and the Northern Great Plains, including Montana (McClay et al., 1999). Due to the success of *A. malherbae* in certain areas of North America, the mite was subsequently released across much of the western United States and also in South Africa (Craemer, 1995) and Mexico (Rodriguez-Navarro et al., 2004).

Eriophyid mites, *A. malherbae* included, have short hemimetabolic life cycles, producing eggs both sexually and asexually via arrhenotokous parthogenesis capable of producing multiple generations within a single season (Helle & Wysoki, 1996). *A. malherbae* produces a leaf gall, a deformity of the plant along the mid-vein of the leaf which provides food and shelter for the mites and eggs. The phenology of the mite coincides with its long-lived perennial hosts so that multiple generations can continuously re-infect the same host. Although not confirmed in *A. malherbae*, within *Aceria cladophthirus* (Nalepa) populations, a single gravid female was able to induce gall development on detached leaves of *Solanum dulcamara L.*, with immature mites also inducing galls to a lesser degree (Westphal 1990). Even if one mite can induce the physical galling process, multiple generations and a heavy mite load are necessary to impact the field bindweed populations. Heavily infested bindweed plants cannot flower or produce seed (Lauriault et al., 2002). *Aceria malherbae* overwinters on root buds just below the soil surface (Ueckermann, 2010); and may also be present on root buds during the growing season (Littlefield per. comm.).

Dispersal capabilities are important in understanding *A. malherbae* colonization and distribution. Eriophyids may be behaviorally adapted to wind dispersal since they move to plant tips and position themselves to be carried away (Michalska et al., 2010; Nault & Styer, 1969; Sabelis & Bruin, 1996; Smith et al., 2010). This behavior occurs to a greater extent on less compatible hosts, aiding dispersal probability (Michalska et al., 2010; Skoracka et al., 2007). Because they are so small, they can also be carried by other insects such as aphids and bees (Sabelis & Bruin, 1996); although phorecy is suspected to be quite rare (Skoracka et al., 2010; Zhao, 2000).

Eriophyid Damage

Eriophyid mites are grouped into two types: free-living (or vagrant), such as *Aceria tulipae*, and gall formers, such as *A. malherbae*. The free-living or vagrant mites create visible feeding damage but do not change host morphology and biochemistry to the extent of gall mites. Nearly half of the 3,700 described eriophyid species are gall formers (Petanović & Kielkiewicz, 2010b). Gall induction changes the nature and development of plant cells, thus gall inducing eriophyid mites are generally both host and tissue specific (Skoracka et al., 2010).

Eighty percent of eriophyids have been reported on only one host species, 95% on only one host genus and 99% on one host family (Skoracka et al., 2010). Some eriophyid mites are so host and tissue specific that they are found to feed only on specific plant biotypes (Skoracka et al., 2010). Because of this intimate relationship between the gall-inducing arthropod and its host, mites such as *A. malherbae*, have been identified as excellent prospects as biological control agents of weeds (Skoracka et al., 2010; Smith et al., 2010). Since the 1970s when eriophyids were first seriously considered for biological control, 70 eriophyid species on 56 plants have been identified for possible use; however, only thirteen specialist eriophyids have been evaluated or released for management of invasive weeds between 1996 and 2010 (Smith et al., 2010).

Eriophyid mites have short feeding stylets (7-30 µm in length) and can only directly impact and feed on epidermal cells just below the leaf cuticle (Royalty & Perring, 1996; Sabelis & Bruin, 1996). However, such feeding kills epidermal cells, distorts plant and leaf growth by cellular enlargement (also known as hypertrophy), and creates structural malformations or changes in mesophyll organization and reduces gas exchange and photosynthesis (Sabelis & Bruin, 1996). This cellular damage occurs with both vagrant and gall-producing feeders, indicating that it is the physical penetration of the stylet, and subsequent chitin introduction into the cell, that causes the cellular damage. The visible cellular responses are directly attributable to the stylet penetration into a single plant cell (Westphal & Manson, 1996). Within 20 minutes of feeding initiation, a callose response, which is a polysaccharide responsible for repair of the cell wall, is evident (Petanović & Kielkiewicz, 2010a; Westphal & Manson, 1996). Callose is both an important component of plant damage and a precursor to gall formation (Petanović & Kielkiewicz, 2010a; Westphal & Manson, 1996). Callose development is also the non-specific response induced by pathogen infection, various abiotic stresses, and mechanical wounding (Nims et al., 1967; Skalamera & Heath, 1996; Westphal & Manson, 1996). Along with callose production, cell vacuoles become alkaline, which affects cell membrane permeability, and the nucleus swells and moves centrally (Westphal & Manson, 1996). Structural change corresponds to a gradual DNA denaturation associated with chitinous accumulation, introduced at the time of initial feeding by the mite (Petanović & Kielkiewicz, 2010a; Westphal & Manson, 1996).

Additionally, an inductive signal is released by the plant during the initial stages of damage during feeding by gall-forming eriophyids (Westphal & Manson, 1996). Feeding enzymes cause a formation of nutritive tissue in the developing gall, which is fed upon by the mites for a specific period according to their life cycle. In the case of *A. malherbae*, extra cellular growth occurs in the mesophyll layer of the field bindweed leaf and results in a doubling in the leaf's mesophyll in comparison to uninfested leaves (Rančic´ & Petanovic´, 2002). This hypertrophy occurs predominantly along the leaf venation and provides the nutritive tissue layer on which the mites feed (Rančic´ & Petanovic´, 2002).

Factors Affecting Biological Control Establishment

Several factors, such as habitat composition, host genetics, and natural enemies of the target species can influence establishment success of biological control arthropod species (Day and Urban 2003). Microclimate as a result of associated habitat of an introduced biological control agent can also have an effect on the ability of a biological control agent to establish. Ozman and Goolsby (2005) studied the development and temperature thresholds of the eriophyid mite *Floracarus perrepae* Knihinicki and Boczek, a proposed biological control agent for the Old World climbing fern (*Lygodium microphyllum* (Cavanilles) R. Brown). Though temperature and humidity were important predictors of these mites' longevity and reproductive capability in the lab, the peak populations in the field did not follow a direct seasonal pattern. Ozman and Goolsby (2005) realized that the leaf rolling created by the mites was a means of increasing humidity and creating a microclimate where the mites could maintain a high reproductive capacity independent of the fluctuating external climate. Clark et al. (2001) also found that the habitat, particularly the size and continuity of knapweed sites, was consistently associated with two knapweed biological control agents' presence or absence within knapweed infested sites. In particular, continuous and nonlinear patches of spotted knapweed on loamy soil that were surrounded by even-age forest stands had the highest rates of *Agapeta zoegnoa* L. (Lepidoptera: Cochylidae) establishment while *Chyphocleonus achates* (Fahraeus) (Coleoptera: Curculionidae) established better in mid elevation (750-1500 m) spotted knapweed infestations greater than two hectares that were continuous and non-linear (Clark et al., 2001).

Plant genetics also play a role in the ability of introduced agents to select, survive, and reproduce on potential hosts. *Aceria chondrillae* (Canestrini), biological control agent for rush skeletonweed, preferentially feeds and develops on select biotypes (Campanella et al., 2009; Caresche & Wapshere, 1974). Ozman & Goolsby (2005) identified distinct biotypes of *F. perrepae* . While these mites more readily induced galls on its native host from Queensland, Australia, they exhibited a diminished ability to induce galls on Old World climbing fern biotypes that were introduced into Florida (Freeman et al., 2005; Ozman & Goolsby, 2005). Freeman et al. (2005) determined that no morphological or anatomical feeding site characteristics could account for the differential galling capabilities and speculated a biochemical basis of this resistance. With field bindweed, specific biotype preferences were found in initial host screenings with four biotypes, with New Jersey plants exhibiting the highest *A. malherbae* infestation levels and Nebraska plants the lowest (Rosenthal & Platts, 1990).

Weed management practices may also influence *A. malherbae* establishment and performance. Widespread herbicide use in croplands, rangelands, railroad rights of way, and roadsides often overlap with regions where *A. malherbae* is established. Even if direct herbicide applications are not intended, indirect impact of commonly used herbicides can occur because of drift (Arvidsson, Bergstrom, & Kreuger, 2011; Kleijn & Snoeijing, 1997; Marrs, Frost, & Plant, 1991). Gall induction by *A. malherbae* is due to the mite modifying cellular development (Petanović & Kielkiewicz, 2010a). Similarly, herbicides alter biochemical pathways and may antagonize or synergize herbivore/weed interactions (Messersmith & Adkins, 1995). For example, sugarcane borer infestations increased following 2,4-D spray and aphid populations increased when oats were treated with with 2,4-D (Adams & Drew, 1969; Ingram et al., 1947; Messersmith & Adkins, 1995). Glyphosate sprayed on wheat curl mite (*Aceria tosichella* Keifer) infested plants also resulted in an immediate increase of the wheat curl mite, though the population peak which occurred by the third day of spray was diminished by the tenth day (Jiang et al., 2005). No effect of herbicides on galls and mites was seen by Boydston and Williams' (2004) study on *Aceria malherbae* and sublethal doses of 2,4-DB or glyphosate.

CHAPTER 2

IMPACTS OF SUB-LETHAL HERBICIDE APPLICATION ON *ACERIA MALHERBAE*, A BIOLOGICAL CONTROL AGENT OF FIELD BINDWEED

Introduction

Convolvulus arvensis L. (field bindweed) is a persistent, perennial weed first brought to the United States in contaminated crop seed (Mitich, 1991). It has an extensive root system and can reproduce both vegetatively and via long-lasting seeds (Frazier, 1943a; b; Timmons, 1949). Field bindweed's viney growth disrupts crop harvest and its extensive root system competes with crops particularly in limited water situations, causing reduced crop yield. Field bindweed also commonly grows along roadsides and in parks. Its problematic presence in 42 countries and 32 different crops, has placed it on the list of the world's worst weeds (Holm, 1977; Jacobs, 2007; Mitich, 1991; Weaver & Riley, 1982). Its native and introduced range extends from 60°N to 45°S; however field bindweed is most problematic within cereal crops in temperate zones (Holm, 1977; Weaver & Riley, 1982).

Field bindweed‘s widespread environmental and economic damage is often reduced with herbicides (Kansas Department of Agriculture, 2006; Peachey, 2012). Resistance or differential susceptibility of field bindweed to herbicides has been documented (Heap, 2012; Powles & Preston, 2006; Westwood & Weller, 1997; Whitworth & Muzik, 1967), therefore an understanding of herbicide modes of action is necessary to minimize multiple and cross resistance (Mallory-Smith & Retzinger, 2003).

Herbicides commonly used on field bindweed in the United States belong to three modes of action: synthetic auxins (group 4), inhibitors of acetolactate synthase (ALS) (also called acetohydroxyacid synthase (AHAS) group 2), and inhibitor of 5-enolypyruvyl-shikimate-3- phosphate synthase (EPSP) (group 9) (Peachey, 2012). Herbicides in the mitosis inhibitor (group 3) and Photosystem I inhibitor (group 22) groups are less commonly used on field bindweed (Kansas Department of Agriculture, 2006; Wright, 2011). Herbicides not commonly recommended for bindweed control, including Photosystem II inhibitors (group 5), inhibitors of protoporphyrinogen oxidase (group 14), inhibitors of cell wall synthesis (groups 21 and 22), and glutamine synthesis inhibitors (group 10), are also known to have some effect on seedling or mature field bindweed plants (Agamalian et al., 2012; Jemison et al., 2007).

Biological control is an additional management tactic for field bindweed management. The objective of biological control is to release natural enemies of the target weed to reduce biomass and/or decrease continued spread of the target weed (McFadyen, 1998). Two biological control agents for field bindweed have been approved for release in the United States, including a noctuid moth, *Tyta luctuosa* Denis & Schiffermuller and the gall mite, *Aceria malherbae* Nuzzaci (Acari: Eriophyidae) (Andreas et al., 2012; Littlefield, 2004).

The eriophyid mite, *A. malherbae* induces galls on field bindweed leaves, with severe infestations stunting plants. *Aceria malherbae* has been established across much of the western United States, and in Texas and New Mexico the mite has reduced above ground biomass up to 90% (Smith et al., 2010). Its effectiveness throughout the Pacific

Northwest of the United States is somewhat more nebulous, with differential rates of mite establishment observed among field bindweed populations or sites, and with control ranging from good to fair (Andreas et al., 2012).

Neither herbicide nor biological control techniques are completely effective alone for field bindweed management. Field bindweed is difficult to control, with most herbicides providing only partial suppression (Boydston & Williams, 2004; Ogg, 1975; Westra et al., 1992) and biological control of field bindweed is slow to be effective in annual crops (Boydston & Williams, 2004). An integrated approach for field bindweed management may be necessary to control this weed over multiple land use situations. Widespread herbicide use in croplands, rangelands, railroad rights of way, and roadsides often overlap with regions where *A. malherbae* is commonly established. Even if direct herbicide applications are not intended, indirect impact of commonly used herbicides can occur because of drift (Arvidsson et al., 2011; Kleijn & Snoeijing, 1997; Marrs et al., 1991).

Gall induction by *A. malherbae* is due to the mite modifying cellular development and biochemical pathways (Petanović & Kielkiewicz, 2010a). Similarly, herbicides alter biochemical pathways and may antagonize or synergize herbivore/weed interactions (Messersmith & Adkins, 1995). For example, populations of the sugarcane borer (*Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae)) and bird cherry-oat aphid (*Rhopalosiphum padi* (L.) (Homoptera: Aphididae)) increased following 2,4-D. Additionally, English grain aphid (*Macrosiphum avenae* (Fabricius) (Homoptera: Aphididae)) populations increased when oats and barley were treated with with 2,4-D

(Adams & Drew, 1969; Ingram et al., 1947; Messersmith & Adkins, 1995). Glyphosate sprayed on wheat curl mite (*Aceria tosichella* Keifer) infested plants resulted in increased mite populations, although the population peak which occurred by the third day diminished by the tenth day (Jiang et al., 2005).

Herbicides that could affect *A. malherbae* are from several different modes of action. Glyphosate was identified as having low or no known adverse effects on *A. malherbae,* according to Boydston and Williams (2004). The ALS enzyme targeted by imazapic might impact gall induction differently than glyphosate. Picloram, like the 2,4-DB tested by Boydston and Williams (2004) is an auxinic herbicide recommended for use on field bindweed with residual soil effects which makes it very likely to contact *A. malherbae* infested field bindweed as it is used on many of the most commonly treated noxious weeds in Montana. Since atrazine is commonly used on large expanses of land throughout the United States (Stackelberg et al., 2012), it is likely to interact with *A. malherbae* infested field bindweed.

Hypotheses and Objectives

Boydston and Williams (2004) studied the combined response of field bindweed to herbicide applications with either 2,4-DB or glyphosate and *A. malherbae* infestation. They observed a synergistic effect in the reduction of field bindweed biomass when management strategies were combined. To more broadly investigate this interaction, a wider range of herbicide sites of actions rather than specific dose responses to a single herbicide is warranted. One purpose of this study, therefore, was to identify and test a wider range of herbicide sites of action than were initially targeted in Boydston and William's (2004) study.

The objective of this study was to determine the impact of sub-lethal herbicide applications on field bindweed plants in combination with *A. malherbae*. I expected to see the combined treatment of *A. malherbae* and glyphosate and other herbicides to produce more growth impact than either *A. malherbae* or herbicide alone, similar to those found by Boydston and Williams (2004). I was interested in evaluating these impacts for herbicides with other modes of action because these other modes of action had not been tested in combination with *A. malherbae* use. Furthermore, I suspected that sub-lethal herbicide use would adversely impact gall induction and therefore mite numbers.

Materials and Methods

Plant Care

Field bindweed plants used in this study were collected as seeds from Phillips County, Montana (Charles M. Russell National Wildlife Refuge 47.62°N 108.59°W) in August 2011. Seeds were scarified by submerging in sulfuric acid for 20 min prior to rinsing with water, and transferring into 9x9x10 cm pots containing Sungro Horticulture Sunshine Mix # 1®, a potting soil formulated with peat moss, starter nutrient and limestone. Seedlings were inoculated with *A. malherbae* collected from Yellowstone County, Montana (45.93°N 108.22°W). The mites were transferred in plant material consisting of field bindweed galls and stored in a refrigerator at approximately 2°C (Britten et al., 2003) over the course of the week-long inoculation process. Two weeks after germination, when seedlings were approximately four cm tall, 25 late instar nymphs or adult mites were hand-transferred to one or two leaves at the apical tip of each plant. All of the plants were maintained at constant 25˚C and 16:8 (light: dark) photoperiod in an environmental chamber for one month. Galled and uninoculated (control) plants were randomly assigned to herbicide treatments. Inoculated plants that did not develop the leaf malformation indicative of a gall were excluded from the study.

Experimental Design and Measurements

The treatment design was a factorial arrangement of two *A. malherbae* treatments (two levels: presence and absence) and six herbicide treatments. The six herbicide treatments included atrazine (Atrazine 4L®), glyphosate (Roundup Original Max®), picloram (Tordon 22K®), imazapic (Plateau®), petroleum crop oil concentrate (Rigo Oil Concentrate®), and water. Plants with low and high gall counts, indicating low and high mite numbers respectively, were dispersed throughout the treatments with six replicates per treatment combination.

Herbicides were applied with a Generation III (DeVries Manufacturing®) spray chamber, fitted with an 8002 even flat fan nozzle and 276 kPa of pressure calibrated for the 286 l/ha application rate to deliver a boom spray of 280, 397, 44, and 210 g a.i./ha for atrazine, glyphosate, imazapic, and picloram respectively based on recommended spray rates for each of the herbicides (Table 2-1). The plants were sprayed with 25% (v/v) of the label recommendation for use on field bindweed, to approximate documented sub-lethal drift exposure to plants (Marrs, 1989). For atrazine, which is not recommended for use on field bindweed, the post emergence rate for annual morning glory (*Ipomoea* sp.)

was utilized. Where a range of rates was recommended, the midpoint of the range was chosen. A water control was used in addition to a petroleum crop oil control treatment which was included because atrazine and imazapic are typically sprayed with a petroleum crop oil adjuvant at 1% v/v.

After herbicide application, plants were arranged in a completely randomized block design in a greenhouse maintained at 20 ˚C (range from 16 C to 27.9 ˚C) and 16:8 (light: dark) photoperiod. The plants were grown for an additional four weeks to allow for adequate expression of treatment effects. Plants were rotated weekly to avoid differing temperature and light patterns within the greenhouse. The plants were watered as needed and fertilized with a 20-20-20 water soluble fertilizer (Scotts ®) twice over the course of the study. The fungicide Strike 50 WDG®, known to have low impact to mites (Childers et al., 1996) was applied to control a powdery mildew problem towards the end of the experiment prior to final harvest.

Table 2-1. Herbicide Recommended Rates and 25% Reduced Rates for Herbicide Application on Field Bindweed

Herbicide	Label Rates of Formulated Product (l/ha)	Reduced Formulated Product Rate (25%) (l/ha)	Active Ingredient (g a.i./ha)	Adjuvant*
Atrazine	2.34	0.59	280	1
Glyphosate	1.61-3.22	0.60	397	0
Imazapic	0.58-0.88	0.18	44	1
Picloram	2.34-4.67	0.88	210	0

* 1 = Rigo Crop oil concentrate at 1% v/v

Measurements

Prior to spraying, the number of leaves, branches, and galls, as well as plant height (cm) were recorded for all inoculated and uninoculated plants. Two weeks after herbicide application, plants were re-measured for plant parameters and number of *A. malherbae* galls. Leaf necrosis and chlorosis, and epinasty were also noted.

Plants were harvested four weeks post application. Final measurements consisted of plant height (cm), number of leaves (separated into live, yellow, dead, and galled categories), and number of branches. Total stem length (cm) and number of ramets were also recorded. Above ground and root biomass (g) were recorded after oven drying at approximately 49-50° C for a minimum of three days. Population levels of *A. malherbae* were determined separately for each gall by dissecting the gall under a dissecting microscope and rating populations by the following categories: 1: 0-5 mites or eggs; 2: 6-25 mites or eggs; 3: 26-75 mites or eggs; 4: > 75 mites or eggs.

Statistical Analysis

Plant height, total stem length, number of branches, total leaves, and above ground, root and total plant biomass were subjected to a two-way ANOVA in R (R Development Core Team, 2011). Variables were assessed for constant variance and normality of residuals. The number of branches and leaves were log transformed prior to analysis of variance. When ANOVA results were significant, linear model and Fisher's Least Significant Difference tests were conducted to determine differences among means at the p=0.05 level (de Mendiburu, 2012). Changes in gall abundance and the proportion of leaves galled were compared between herbicide treatments for inoculated plants using Kruskal-Wallis nonparametric tests (R Development Core Team, 2011).

Results

Interaction Between Herbicide and *A. Malherbae* Inoculation

There was no significant interaction between herbicide and *A. malherbae* for any of the plant parameters measured (Table 2-2, means Table 2-3).

Table 2-2. Significance (P) of Treatment Interactions, Herbicide, and *Aceria Malherbae* on Plant Height, Total Stem Length, Number of Branches, Total Leaves, and Above Ground, Root and Total Plant Biomass of Field Bindweed Plants

	Plant Height (cm)	Stem Length (cm)	Branches (no.)	Total Leaves (no.)	Ramets (no.)	Above Ground Biomass (g)	Biomass Root (g)	Biomass Total (g)
Herbicide * Mite	1.40 (0.24)	0.58 (0.72)	0.87 (0.51)	0.72 (0.61)	0.50 (0.79)	1.13 (0.35)	0.57 (0.73)	1.10 (0.37)
Herbicide	1.55 (0.19)	5.19 (<0.00)	10.56 (<0.00)	5.50 (<0.00)	17.05 (<0.00)	3.40 (0.01)	2.56 (0.04)	3.92 (0.00)
Mite	0.18 (0.67)	8.23 (0.01)	16.28 (<0.00)	2.42 (0.12)	0.75 (0.39)	5.45 (0.02)	0.10 (0.75)	4.58 (0.04)

Herbicide Effects on Field Bindweed

Main effects for herbicide were significant for all variables except for plant height (Table 2-2). The oil control group did not significantly differ from the water control group for any of the growth parameters measured (Table 2-4). In general, sub-lethal applications of herbicides did not have an adverse effect on stem height, total stem length, numbers of leaves or branches, or on above-ground or below-ground biomass compared to the control groups. However, glyphosate as compared to water control reduced above ground biomass by 21% (T=-2.097, P=0.039). Imazapic reduced total stem length by 34% (T=-3.45, P<0.0001). Imazapic also reduced the number of branches by 28% (T = -2.284, P = 0.025). Picloram had the greatest effect on plant growth parameters with epinastic stems evident within one week of spray treatment, but picloram did not affect plant height or root biomass and decreased total stem length by 34% (T=-3.337, P=0.001). Picloram also reduced the number of branches (T=-5.125, P<0.0001) and leaves (T=-4.028, P=0.0001) by 50%. Above ground biomass was 36% less in picloram treated plants compared with the control (T=-3.501,P=0.022) and total biomass reduced by 32% (T=-3.796, P=0.0003) (Table 2-4).

Aceria Malherbae Effects on Field Bindweed

Gall mite effects were significant across fewer variables than the herbicide effects (Table 2-2). The presence of mites reduced total stem length by 16% compared to uninoculated controls (T=-2.91, P=0.0047) (Table 2-5). *Aceria malherbae* also reduced branching of field bindweed by 27% (T=-4.053, P=0.0001). In addition both above and below ground biomass were impacted (T=-2.324, P=0.023; T=-2.134, P=0.036, respectively), with a 13% decrease in above ground biomass.

Field bindweed plants inoculated with *A. malherbae* had a mean of 11 (± 1.5) galls per plant prior to spray and a mean of 37 (± 8.1) galls at harvest (Table 2.6). Two uninoculated plants had galls before the third week of the study and these galls were manually removed. The change in gall numbers from pre-application to harvest did not differ among herbicide treatments (P= 0.22, DF=5) (Table 2-6); nor did the proportion of leaves galled (P=0.53, DF=5) (Table 2-6). The number of mites and eggs also were not affected by herbicide use at the non-lethal rate tested, despite cases of significant loss of leaves such as in the picloram treatment (Table 2-5).

Table 2-3. Growth Response[1] of Field Bindweed to Low Level Herbicide and Inoculation Treatments

Mites	Herbicide	Plant Height (cm)	Total Stem Length (cm)	Branches (no.)	Total Leaves (no.)	Ramets (no.)	Above Ground Biomass (g)	Root Biomass (g)	Total Biomass (g)
Present									
	Atrazine	66.07 (± 2.02)a	504.54 (± 35.28)ab	22.6 (± 3.6)abc	218.4 (± 36.1)ab	4.6 (± 1.6)ab	5.31 (±0.82)a	1.63 (±0.22)	6.94 (±0.76)ab
	Glyphosate	59.29 (± 2.34)a	506.93 (± 53.92)ab	27.7 (± 2.3)abc	193.9 (± 20. 7)ab	7.7 (± 1.7)ab	4.27 (±0.52)a	2.09 (±0.11)	6.36 (±0.52)ab
	Imazapic	54.29 (± 6.86)a	330.64 (± 42.64)ab	17.4 (± 1.3)abc	192.0 (± 26.7)ab	1.0 (± 0.0)ab	5.04 (±0.45)a	1.60 (±0.17)	6.64 (±0.44)ab
	Oil	58.29 (± 3.64)a	393.91 (± 26.34)ab	17.4 ± 1.0)abc	173.1 (± 15.3)ab	4.6 (± 0.8)ab	4.33 (±0.52)a	1.87 (±0.16)	6.20 (±0.57)ab
	Picloram*	46.86 (± 4.89)a	280.86 (± 33.42)b	11.0 (± 1. 5)c	98.0 (± 12.4)b	1.1 (± 0.1)b	2.78 (±0.25)a	1.21 (±0.15)	3.99 (±0.35)b
	Water	42.20 (± 5.74)a	497.42 (± 48.53)ab	21.2 (± 2.2)abc	236.8 (± 33.0)a	3.2 (± 1.0)ab	5.40 (±0.57)a	1.73 (±0.20)	7.13 (±0.54)ab
Absent									
	Atrazine	55.34 (± 4.03)a	594.79 (± 81.04)a	33.1 (± 4.2)a	307.3 (± 44.9)a	4.4 (± 1.0)ab	5.38 (±0.85)a	1.58 (±0.23)	6.96 (±0.79)ab
	Glyphosate	51.14 (± 6.33)a	540.21 (± 68.37)ab	33.1 (± 4.6)abc	240.9 (± 33.5)a	6.0 (± 1.0)ab	4.72 (±0.52)a	1.86 (±0.23)	6.58 (±0.65)ab
	Imazapic	52.70 (± 3.60)a	362.00 (± 60.41)ab	21.4 (± 4.0)abc	171.9 (± 19.8)ab	2.3 (± 1.1)ab	4.89 (±0.78)a	1.73 (±0.22)	6.62 (±0.94)ab
	Oil	54.83 (± 6.69)a	592.49 (± 61.43)a	33.0 (± 3.8)abc	215.6 (± 27.1)ab	4.4 (± 0.9)a	6.54 (±0.71)a	1.74 (±0.16)	8.28 (±0.71)a
	Picloram	56.25 (± 4.16)a	421.68 (± 38.36)ab	14.7 (± 2.3)bc	132.0 (± 15.3)ab	1.0 (± 0.0)ab	4.44 (±0.49)a	1.49 (±0.14)	5.93 (±0.52)ab
	Water*	51.56 (± 7.17)a	610.89 (± 113.08)a	34.9 (± 6.5)ab	265.9 (± 62.4)ab	6.1 (± 2.5)a	6.21 (±0.59)a	1.54 (±0.19)	7.75 (±0.66)a

[1]Means with the same letter are not significantly different by Bonferroni multiple comparison test at the p=.05 level
Each value is the mean of 6 plants
* 5 plants made up this mean

Table 2-4. Growth Response[1] of Field Bindweed to Main Effects of Low Level Herbicide Pooled over Inoculation Treatment

Herbicide Treatment	Plant Height (cm)	Total Stem Length (cm)	Branches (no.)	Total Leaves (no.)	Ramets (no.)	Above Ground Biomass (g)	Root Biomass (g)	Total Biomass (g)
Atrazine	60.7 (± 2.6)	549.7 (± 44.3)	27.9 (± 3.02)	282.9 (± 30.3)	4.5 (± 0.91)	5.35 (± 0.57)	1.60 (± 0.15)	6.95 (± 0.53)
Glyphosat	55.2 (± 3.4)	523.6 (± 42.1)	30.4 (± 2.69)	217.4 (± 20.0)	6.9 (± 0.95)	4.50 (± 0.36)	1.97 (± 0.13)	6.47 (± 0.40)
Imazapic	53.6 (± 4.1)	346.3 (± 35.8)	19.4 (± 2.10)	181.9 (± 16.2)	1.6 (± 0.57)	4.96 (± 0.44)	1.66 (± 0.14)	6.63 (± 0.50)
Oil	56.7 (± 3.5)	493.2 (± 42.3)	25.2 (± 2.86)	194.4 (± 16.1)	4.5 (± 0.59)	5.43 (± 0.52)	1.80 (± 0.11)	7.24 (± 0.52)
Picloram*	51.2 (± 3.4)	345.9 (± 31.5)	12.7 (± 1.36)	113.7 (± 10.5)	1.1 (± 0.08)	3.54 (± 0.34)	1.34 (± 0.11)	4.88 (± 0.40)
Water*	47.7 (± 4.8)	558.5 (± 64.5)	28.5 (± 4.02)	252.5 (± 35.7)	4.8 (± 1.44)	5.84 (± 0.41)	1.63 (± 0.14)	7.47 (± 0.42)

[1]Each value is the mean of 12 plants
* 11 plants made up this mean

Table 2-5. Growth[1] of Field Bindweed to Main Effects of Inoculation Treatments Pooled over Herbicide Treatment

Mite Treatment	Plant Height (cm)	Total Stem Length (cm)	Branches (no.)	Total Leaves (no.)	Ramets (no.)	Above Ground Biomass (g)	Root Biomass (g)	Total Biomass (g)
Present	55.1 (± 2.1)	417.1 (± 21.1)	19.5 (± 1.2)	184.1 (± 11.9)	3.7 (± 0.6)	4.5 (±0.3)	1.7 (±0.1)	6.2 (±0.3)
Absent	53.6 (± 2.2)	522.8 (± 32.5)	28.7 (± 2.1)	224.4 (± 17.2)	4.1 (± 0.6)	5.4 (±0.3)	1.7 (±0.1)	7.1 (±0.3)

[1]Each value is the mean of 36 (± SE)

Table 2-6. *Aceria Malherbae* Gall and Mite Response to Herbicide Treatments

Herbicide	Change in Proportion of Leaves Galled+	Change in No. Leaves Galled+	Mite No. Rating**	Egg No. Rating**
Atrazine	0.06 (± 0.06)a	30.0 (± 19.0)a	1.8 (± 0.1)	1.5 (± 0.1)
Glyphosate	- 0.02 (± 0.04)a	3.9 (± 9.2)a	1.1 (± 0.0)	1.1 (± 0.1)
Imazapic	0.13 (± 0.09)a	39.4 (± 17.1)a	2.0 (± 0.1)	1. 8 (± 0.1)
Oil	0.06 (± 0.04)a	19.4 (± 11.5)a	1.6 (± 0.1)	1.5 (± 0.1)
Picloram*	0.01 (± 0.02)a	0.1 (± 1.2)a	1.7 (± 0.1)	1.6 (± 0.1)
Water*	0.10 (± 0.08)a	44.8 (± 24.2)a	1.6 (± 0.2)	1.5 (± 0.1)

[1] Each value is the mean of 12 (± SE)
*11 plants make up this mean
+Change in galls = final no. leaves galled / total no. leaves – pre spray no. leaves galled / total no. leaves
**Rating Category: 1: 0-5 mites and 0-5 eggs 2: 6-25 mites and eggs 3: 26-75 mites and eggs 4: 75 mites and eggs

Discussion

Variable Responses of Plants to Herbicide Treatment

The low levels of herbicide used in this study mimicked drift effect levels and were not outside of expected ranges as reported in the literature (Davis et al., 1994; Kleijn & Snoeijing, 1997; Marrs et al., 1989). Kleijn & Snoeijing (1997) found that when herbicides were sprayed at below 50% of label recommended rates, herbicidal effects varied significantly across years and planting environments. The effects of low level herbicides have been variable in many greenhouse and field experiments, with different species and vigor of the plants affecting drift-level response to herbicides (Kleijn & Snoeijing, 1997). Thus, it stands to reason that minor differences between the study of low-level herbicide use on field bindweed conducted by Boydston and Williams (2004) and this study exist. Some plant species display morphological effects, such as epinasty, after exposure to herbicides, while other effects are more subtle or cannot be visually detected. Davis et al. (1994) also reported that herbicide impact depended on the target's sensitivity and were nonlinear; thus making low-level responses difficult to determine. Even at recommended rates, such as in trials used to determine the lethal dose levels needed to manage field bindweed, plant response was variable (Schoenhals et al., 1990; Westwood & Weller, 1997). For example, Schoenhals et al. (1990) found that field bindweed control with recommended rates of imazapyr only exceeded 50% in 5 out of the 16 trials; the authors attributed this to plant vigor. Variable responses of the plant based on the duration and intensity of herbicidal treatments have been observed as well (Kleijn & Snoeijing, 1997; Marrs et al., 1991). For example, simulated drift effects of low level fluroxypyr treatments were detected by Kleijn and Snoejing (1997) almost immediately after application, yet after six weeks, the differences between treatment and control plants were barely detectable; perhaps indicating that recovery rates also differed at these low levels. Kleijn and Snoejing (1997) also noted that treatment effects could also be expressed gradually in some situations. For example, although no immediate extinction occurred in some of Kliejn and Snoejing's (1997) field studies, they noted that treated plants could have had depleted resources that increased winter mortality. Marrs et al. (1991) noted similar concern over short greenhouse bioassays. Kleijn and Snoeijing (1997) as well as Marrs et al. (1991) claim that compensatory effects in the field typically were more relevant than greenhouse bioassays which terminate before plant response can be accurately measured.

Proposed Phenological Impact on Synergism Between Herbicide and *A. Malherbae*

Glyphosate and *A. malherbae* were synergistic, causing greater field bindweed biomass reduction together than either low-level glyphosate or *A. malherbae* could cause alone (Boydston & Williams, 2004). The synergistic responses found by Boydston and Williams (2004) were not replicated in this study. The phenological stage of field bindweed could affect plant response to herbicides or *A. malherbae*. In the University of California Davis' herbicide susceptibility database (Agamalian et al., 2012), the effects of various herbicides on field bindweed are grouped according to plant stage (seedling or perennial). Typically young and small plants are more susceptible than older plants to herbicides (Roberts, 1982 in Marrs et al., 1991). Since rhizomes were used for test plant establishment in Boydston and Williams' (2004) study and seedlings were used for this study, differential treatment responses may have occurred due to plant stage. Similarly, galls developed over the course of one month prior to spraying in this study as compared to a much shorter gall development time of eight to nine days in Boydston and Williams' (2004) study; therefore, the gall and mite stage could also influence any synergism between *A. malherbae* and herbicide treatments previously found.

Implications for Management

The herbicide and mite levels used in this study were without apparent antagonistic or synergistic affects; this result differs from Boydston and Williams (2004). It is possible that a broader range of herbicide concentrations and mite levels might identify the range in which the combined effects of the two treatments begin to proliferate. No evidence of herbicide impact on gall or mite production was identified across the mode of action groupings tested. These findings indicate that the gall induction pathways are tolerant to a wide range of herbicides and provide managers justification for the use of herbicide simultaneously with *A. malherbae*, especially in situations where herbicide resistance may be of concern. Drift is the most likely scenario where *A. malherbae* would be used where herbicides are also being used, therefore this research provides further evidence that these low levels of herbicide will not negatively impact *A. malherbae* gall induction on field bindweed. However, increased plant damage as a result of higher herbicide application rates could reduce the number of leaves or apical tips for gall induction by *A. malherbae* (Manalil et al., 2011; Yu et al., 2013), and intentional low-level herbicide use could increase the likelihood of herbicide resistance evolving (Manalil et al., 2011; Yu et al., 2013).

CHAPTER 3

INFLUENCE OF WESTERN UNITED STATES FIELD BINDWEED POPULATIONS ON *ACERIA MALHERBAE* GALL INDUCTION

Introduction

Invasive species cause widespread economic losses throughout the United States; currently estimated at almost 120 billion dollars per year (Pimentel, 2005; 2009; Pimentel et al., 2005). In addition to economic impacts, invasive species threaten the integrity of many ecological systems resulting in the loss of biodiversity (Pimentel, 2005; 2009; Pimentel et al., 2005). Field bindweed (*Convolvulus arvensis* L.) is one of these problematic invasive species.

Field bindweed was brought to the United States as early as 1739 from Europe and Asia in contaminated crop seed (Weaver & Riley, 1982). By 1908, field bindweed was already recognized as a problem plant capable of reducing crop yield. It has tenacious rhizomes that can spread the plant vegetatively and seeds can reside in the soil seed bank for decades (Timmons, 1949). Due to these invasive characteristics, field bindweed has been able to spread and occupy large expanses of the continental United States and Hawaii (USDA NRCS, 2013).

Field bindweed, like many invasive species and weeds, has been extensively researched for methods to reduce its negative impact. The eriophyid mite *Aceria malherbae* Nuzzaci has been released as a biological control agent to manage field bindweed in Texas (Boldt & Sobhian, 1993) and in the Great Plains, including Montana

(McClay et al., 1999). *Aceria malherbae* in New Mexico and Texas has been successful, particularly in non-crop settings, with as much as 95% biomass reduction of field bindweed being observed; whereas in Montana, field bindweed reduction has been spotty (Smith et al., 2010).

Increased heterozygosity has been highlighted as an important trait that may make plants more invasive (Ellstrand & Schierenbeck, 2000; Lee, 2002). Hybridization (Fritz et al., 1999; Strauss, 1994) and polyploidy, common in plants, have been identified as likely “engines of biodiversity” (Meimberg et al., 2009). Species that are known to hybridize or that are polyploids have higher incidence of heterozygosity (Hartfield et al., 2012; Otto & Whitton, 2000). Heterozygosity allows populations to mask disadvantageous mutations with beneficial dominant traits and also allows for more phenotypic plasticity (Otto & Whitton, 2000). Since field bindweed is an obligate outcrosser (Sherwood & Fuelleman, 1948), the potential for high heterozygosity exists in field bindweed populations. Heterozygosity also creates the potential for distinct biotypes to exist within a population. Biotypes have been identified in field bindweed populations based on leaf shape proportions and flowering differences (Brown, 1946; Degennaro & Weller, 1984).

Eriophyid mites have been investigated as potential biological control agents of invasive weeds due to their high host specificity (Skoracka et al., 2010; Smith et al., 2010). The majority of eriophyid mites are restricted to a single host plant or closely related plant species (Skoracka et al., 2010). However such specificity may extend to the subspecies level. For example, the rush skeletonweed gall mite *A. chondrillae*, feeds and develops on specific *Chondrilla juncea* biotypes (Campanella et al., 2009; Caresche &

Wapshere, 1974; Cullen & Moore, 1983; Sobhian & Andres, 1978). In initial host screenings of *A. malherbae*, (Rosenthal & Platts, 1990) observed differential infestation levels among field bindweed biotypes, with New Jersey plants exhibiting the highest numbers of galls and Nebraska plants the lowest.

Hypotheses and Objectives

The establishment and effectiveness of *A. malherbae* has been reported to vary across western North America (Andreas et al., 2012; McClay et al., 1999; Smith et al., 2010). Factors such as climate and management practices may explain some of this variation. However, the genetic variation of its host, field bindweed, might also be a contributing factor. Therefore, I hypothesized that genetic differences among field bindweed populations found over a large geographic area would result in differential development of *A. malherbae.* Specifically, I hypothesized that: 1) field bindweed populations collected over a large geographic area will exhibit differences in plant architecture and development (i.e. stem height, branching, numbers of leaves, etc.); and 2) the performance of *A. malherbae* (i.e. ability to induce galls and impact the plant) would differ relative to plant population.

Materials and Methods

Plant Propagation

Field bindweed populations were selected from distinct geographic regions throughout the Western United States to increase the chance of greater genetic and biotypic diversity. Seeds were obtained from four sites: Ventura, CA (34.24°N 119.30°W) collected October 2009; Socorro, NM (34.06°N 106.88°W) collected October 2011; Wasco, OR (45.60°N 121.15°W) collected September 2011; and Phillips Co. MT (Charles M Russell National Wildlife Refuge 47.62°N 108.59°W) collected August 2011.

To promote germination, seeds were scarified in sulfuric acid for 20 min prior to rinsing with water, and then transferred into 9x9x10 cm pots containing Sungro Horticulture Sunshine No. 1 Mix® horticultural growing medium. One week after germination, plants were transplanted into 12x12x13 cm pots with plastic trellises which enabled plants to grow without intertwining and provide ease in plant measurements.

Aceria malherbae collected from Reed Point Montana (45.71°N 109.54°W), and Huntley Montana (45.93°N 108.22°W) were used for this study; collected mites were transferred from dissected plant galls to plants within a week of harvest from the growing plant for the first replication. For the second experimental replication, greenhouse reared mites harvested from a variety of plant locations were used to inoculate plants. Thirty-five late instar or adult mites were hand-transferred to one or two leaves at the apical tip of each plant two weeks after germination; when plants were approximately four cm tall. Un-inoculated plants were utilized as a control. Plants were maintained in an environmental chamber at 25˚C, ambient relative humidity and 16:8 (light: dark) photoperiod. All plants were watered daily and fertilized as needed with a 20/20/20 (N/P/K) water soluble fertilizer (Scotts ®) every one to two weeks during the study.

Measurements

Plant height (cm), and number of leaves, branches, and galls were recorded every other week post inoculation until plant harvest. Phenotypic variations in plants such as leaf size and shape were also noted. Ten weeks after inoculation, plants were harvested and plant height, number of leaves, galls and branches were again recorded, along with total stem length (cm). Plant leaves, stems, galls, and roots from each plant were placed in an oven at 49-50˚C and dried for at least three days.

Statistical Analysis

The treatment design consisted of a factorial arrangement of four plant location treatments and two *A. malherbae* treatment levels (present and absent). Plants were arranged in a randomized complete block design with five replications per treatment. The experiment was repeated for a total of ten replications. Plant height, total stem length, number of branches, number of total leaves, and above ground, root and total plant biomass were subjected to a two-way ANOVA mixed models using the nlme package in R (Pinheiro et al., 2012). Variables were assessed for constant variance and normality of residuals and total stem length, number of branches, and leaves were square root transformed and above ground biomass was log transformed prior to analysis of variance. When ANOVA results were significant, a linear model was used to assess which treatments differed from each other (Pinheiro et al., 2012). Gall abundance was compared using a Friedman nonparametric test (R Development Core Team, 2011) and a Friedman multiple comparison test using the agricolae package in R (de Mendiburu, 2012).

Results

Interaction Between Location and *A. Malherbae* Inoculation

No interactions between main and block effects were observed, so data were pooled across experiments. A significant interaction between location and mite was observed in the number of leaves (F=4.81, P=0.01) but not for the other variables (Table 3-1). The unique response of greater leaf production of the New Mexico plants to *A. malherbae* inoculation was evident (Table 3-2) and is discussed below.

Table 3-1. Statistical Significance (P) of Field Bindweed Location, *Aceria Malherbae*, and Treatment Interactions on Plant Height, Total Stem Length, Number of Branches, Total Leaves, and Above Ground, Root and Total Plant Biomass of Field Bindweed Plants

		F-value (P)						
	DF	Plant Height (cm)	Stem Length (no.)	Branches (no.)	Total Leaves (no.)	Biomass Above Ground (g)	Biomass Root (g)	Biomass Total (g)
Location x Mite	3	1.10 (0.36)	1.02 (0.39)	2.66 (0.06)	4.81 (0.01)	1.22 (0.31)	1.97 (0.13)	1.47 (0.23)
Location	3	8.30 (0.0001)	5.35 (0.002)	3.81 (0.02)	3.97 (0.01)	2.58 (0.06)	0.76 (0.52)	5.35 (0.66)
Mite	1	2.50 (0.15)	1.11 (0.30)	7. 71 (0.02)	2.85 (0.13)	0.29 (0.60)	4.53 (0.04)	0.67 (0.41)

Location Effect on Field Bindweed Growth

Location differences were detected for plant height, stem length, and number of branches (Table 3-1 and Table 3-3). Plant height was significantly different among locations (F=8.30, P<0.001) with mean plant height ranging between 59.4 and 73.4 cm (Table 3-3). California plants were taller than both Oregon (F=2.44, P=0.02) and Montana plants (F =-2.6, P=0.01), and the New Mexican plant population was significantly taller than Montana plants (F=4.01, P<0.001).The mean differences in plant height among populations were as much as 25% between the tallest plants, which were predominantly from New Mexico, and the shortest plants, from Montana. Total stem length was also greater for plants from New Mexico than those from California, Oregon, or Montana (F=3.27, P=.002). There was also a main effect for number of branches for locations detected (F=3.81, P=0.02); New Mexico, Montana, and Oregon had more branches than California. There was no location difference in above ground biomass (F=2.58, P=0.06), root biomass (F=0.76, P=0.52) or total biomass (F=5.35, P=0.66) of the plant based on F-test comparisons.

Aceria Malherbae Effects on Field Bindweed

Main effect differences for mite were detected for number of branches (F=7.71, P=0.02) and root biomass (F=4.53, P=0.04) (Table 3-1). For plant without and with mites, respectively, mean number of branches, ranged between 15.3 and 19.4 and mean root biomass ranged from 0.45 g to 0.56 g (Table 3-4). In general, galling by *A. malherbae* did not significantly impact plant height, total stem length, number of branches or total leaves, nor above ground and total plant biomass of field bindweed (Table 3-4).

Gall induction did not occur in ten percent of all inoculated plants. These included two plants from New Mexico, one plant from Montana, and one plant from Oregon. Significant differences in total gall numbers were observed among field bindweed biotypes (Table 3-5). Mean number of galls ranged between 3.6 and 25.0 galls per plant, with the New Mexico biotype having significantly fewer galls compared to Oregon ($\chi^2 = 11.85$, $P<0.01$). The number of mites present within galls was consistently low (<10 adults or nymphs) across treatments (personal observation).

Table 3-2. Growth Response[1] of Field Bindweed by Geographic Locations and Exposure to *Aceria Malherbae* Inoculation Treatments

Mites	Location	Plant Height (cm)	Total Stem Length (cm) *	Branches (no.) *	Total Leaves (no.)*	Above Ground Biomass (g) **	Root Biomass (g)	Total Biomass (g)
Present								
	California	67.3 (± 4.5)ab	316.2 (± 38.5)b	14.7 (± 2.1)b	175.2 (± 19.2)b	1.16 (± 0.14)a	0.35 (± 0.07)a	1.51 (± 0.18)a
	Montana	59.4 (± 2.3)b	343.9 (± 36.1)ab	19.5 (± 2.6)ab	251.5 (± 25.4)ab	0.99 (± 0.09)a	0.54 (± 0.11)a	1.53 (± 0.16)a
	New Mexico	68.0 (± 4.3)ab	516.6 (± 58.1)a	26.7 (± 3.9)a	317.7 (± 37.6)a	1.47 (± 0.13)a	0.43 (± 0.07)a	1.90 (± 0.19)a
	Oregon	60.0 (± 4.1)b	299.7 (± 41.8)b	16.6 (± 1.1)ab	210.0 (± 17.9)ab	1.04 (± 0.10)a	0.48 (± 0.06)a	1.52 (± 0.14)a
Absent								
	California	71.9 (± 3.0)ab	317.8 (± 26.3)b	12.6 (± 1.8)b	187.0 (± 26.2)b	1.27 (± 0.10)a	0.67 (± 0.10)a	1.95 (± 0.18)a
	Montana	59.3 (± 3.1)b	341.0 (± 49.6)ab	17.2 (± 2.2)ab	227.1 (± 27.8)ab	1.04 (± 0.19)a	0.59 (± 0.12)a	1.63 (± 0.28)a
	New Mexico	78.7 (± 3.7)a	390.5 (± 56.2)ab	14.6 (± 2.2)b	178.9 (± 15.8)b	1.17 (± 0.19)a	0.44 (± 0.07)a	1.61 (± 0.24)a
	Oregon	60.1 (± 4.2)b	299.9 (± 43.0)b	16.9 (± 2.7)ab	224.6 (± 32.8)ab	1.11 (± 0.16)a	0.55 (± 0.10)a	1.67 (± 0.23)a

[1]Means represent an average of 10 (± SE); those means with the same letter within a column are not significantly different by T test comparison at the p=0.05 level within mite treatments.
* Square root transformed for analysis due to deviance from normality
** Log transformed for analysis due to deviance from normality

Table 3-3. Growth Response[1] of Field Bindweed by Geographic Population Pooled over Inoculation Treatment

Location	Plant Height (cm)	Total Stem Length (cm) *	Branches (no.) *	Total Leaves (no.) *	Above Ground Biomass (g) **	Root Biomass (g)	Total Biomass (g)
California	69.6 (± 4.3)a	317.0 (± 22.7)b	13.7 (± 1.4)	181.1 (± 15.9)	1.22 (± 0.08)a	0.51 (± 0.07)a	1.73 (± 0.13)a
Montana	59.4 (± 1.9)b	342. 5 (± 29.9)b	18.4 (± 1.7)	239.3 (± 18.5)	1.01 (± 0.10)a	0.57 (± 0.08)a	1.58 (± 0.16)a
New Mexico	73.4 (± 3.0)a	453.5 (± 41.9)a	20.7 (± 2.6)	248.3 (± 25.6)	1.32 (± 0.12)a	0.43 (± 0.05)a	1.75 (± 0.15)a
Oregon	60.1 (± 2.9)b	299.8 (± 29.2)b	16.8 (± 1.4)	217.3 (± 18.2)	1.08 (± 0.09)a	0.52 (± 0.06)a	1.59 (± 0.13)a

[1]Means with the same letter within a column are not significantly different by T test comparison at the p=0.05 level
Each value is the mean of 20 (± SE) plants
* Square root transformed for analysis due to deviance from normality
** Log transformed for analysis due to deviance from normality

Table 3-4. Growth Response[1] of Field Bindweed by Exposure to *Aceria malherbae* Pooled over Geographic Population

Mites	Plant Height (cm)	Total Stem Length (cm) *	Branches (no.) *	Total Leaves (no.) *	Above Ground Biomass (g) **	Root Biomass (g)	Total Biomass (g)
Absent	67.5 (± 2.2)	337.3 (± 22.4)	15.3 (± 1.1)	204.4 (± 13.1)	1.15 (± 0.08)	0.56 (± 0.05)	1.71 (± 0.12)
Present	63.7 (± 2.0)	369.1 (± 25.5)	19.4 (± 1.45)	238.6 (± 15.2)	1.16 (± 0.06)	0.45 (± 0.04)	1.61 (± 0.09)

[1]Each value is the mean of 40 (± SE)
* Square root transformed for analysis due to deviance from normality
** Log transformed for analysis due to deviance from normality

Table 3-5. Gall Induction[1] of *Aceria Malherbae* on Field Bindweed from Geographic Locations

Plant Location	Total Galls
California	10.0 (± 4.0)ab
Montana	13.5 (± 5.2)a
New Mexico	3.6 (± 2.5)b
Oregon	25.0 (± 7.4)a

[1]Means with the same letter are not significantly different by Friedman multiple comparison at the p=0.05 level.
Each value is the mean of 10 (± SE) plants

Discussion

Location and Aceria Malherbae Effects on Field Bindweed

Observed growth parameters suggested population size differences among the plants from distinct locations. The differences in growth parameters, particularly the distinct stem lengths found for the different locations, differed from the leaf and flowering characteristics previously used by Brown et al. (1948) or Degennaro and Weller (1984) to differentiate field bindweed biotypes. In this study, no differences in leaf characteristics were evident among the locations tested (personal observation), and no flowering occurred, making characterization of floral morphology and flowering duration and frequency impossible. Even with the lack of distinct morphological characteristics as described by previous researchers, the measured growth parameters that did differ in a common environment indicate distinct genotypes in field bindweed populations. These growth differences, when used as an indication of genotypic variation among populations might indicate inherent traits that make the plants more or less suitable to mite establishment and development. Michalska et al. (2010) suggested that plant chemical composition, nutritional quality, toxicity, and growth stage, structure, microenvironment and relationship with other herbivores present could all be factors affecting herbivore establishment. Visible plant effects may have been a direct response of the plant to *A. malherbae* feeding. Since the plants grown from seed collected from New Mexico exhibited a difference in number of leaves when *A. malherbae* was present compared to when *A. malherbae* was absent, this may be an indication that the plants were compensating in the presence of *A. malherbae* feeding.

A better understanding of the physiological response of the plant to the feeding by *A. malherbae* and the resulting defenses produced by the field bindweed plants would be needed to determine why the plants are producing more leaves when fed on by *A. malherbae* and why this response is specifically seen in only the population from New Mexico. Other researchers have found chemical or physical defense against herbivory (Boalt et al., 2010). These defense mechanisms include increased growth rate, higher photosynthetic capacity, and changed resource allocation following defoliation (Boalt et al., 2010). Boalt et al. (2010) also found a fitness increase, measured in high seed production in more tolerant populations of the perennial herb *Cardamine pratensis* L. to defoliation by the orange tip butterfly, *Anthocharis cardamines* L.

Implications for Management

Knowledge of field bindweed's susceptibility to *A. malherbae* infestation may help continued management efforts against field bindweed over a wider geographic or genotypic range. Field bindweed populations were already known to have variable *A. malherbae* susceptibility (Rosenthal & Platts, 1990) and in this study, plants collected from New Mexico had less *A. malherbae* gall induction than other populations studied. Whether this variability of susceptibility of field bindweed to the mite will cause selective pressure is not known. However selective adaptation has been documented in biological control. The use of the pathogenic myxomatosis in the formerly successful biological control of rabbits in Australia is one of the classic examples of the development of resistance in biological control (Holt & Hochberg, 1997; Hufbauer & Roderick, 2005). Rush skeletonweed has also shown resistance to a rust biological control agent, *Puccinia condrillina* in Australia (Campanella et al., 2009). However, Holt and Hochberg (1997) suggest there is no clear evidence of an effect on rush skeletonweed populations caused by *P. condrillina*. Documented occurrences of resistance to arthropod biological control agents are fewer, though some evidence does exist, including a 1910 introduction of a sawfly parasitoid (Holt & Hochberg, 1997). When later researchers studied outbreaks of sawfly population, they found the parasitoid eggs encapsulated, indicative of a defense against the parasitoid egg (Holt & Hochberg, 1997; Muldrew, 1953). Some of the researchers suggested that a resistance had evolved; others suggested that resistant sawflies had simultaneously been introduced into the population (Holt & Hochberg, 1997). The selection pressure required to create resistant populations might not be observable in short term biological control studies.

The populations of *A. malherbae* approved for release in the United States, were primarily from one European origin, northern Greece (Boldt & Sobhian, 1993). Goolsby et al. (2006) matched overlapping geographic regions in Australia of the Old World climbing fern and its biological control agent using phylogenetic markers. They found that overlapping geographic regions resulted in increased host susceptibility to the eriophyid mite, *Floracarus perrepae.* Implementing genetic analysis strategies such as Goolsby et al. (2006), could help to improve agent selection or help identify more compatible biological control agents for a wider geographic or genotypic range of the target host. Although genetic analyses were not regularly conducted at the time of the screening of *A. malherbae*, improving biological control agent selection may improve the future success of highly host specific biological control agents such as eriophyid mites or pathogenic fungi. Even though no evidence of field bindweed populations possessing resistance to *A. malherbae* has been reported in the literature, further improvement of host/agent selection could help the successful management of future weed biological control agent efforts.

The interaction between genetics and the environment is key to understanding biological control agent establishment and host plant sensitivity. Genetic effects can, and likely do, play a part, but the much more variable environmental factors that could be helping or limiting mite establishment in the field could have a larger role that would quickly mask any detectable genetic effects. Further experiments to study the relationship between the genetic component of mite establishment and the environmental component might help to clarify this relationship.

CHAPTER 4

FIELD SURVEY OF *ACERIA MALHERBAE*: MITE PRESENCE AND INTER AND INTRA-FIELD SITE HABITAT VARIATIONS

Introduction

Field bindweed (*Convolvulus arvensis* L.) is a perennial, invasive plant with a persistent, deep root system that can spread vegetatively, and with seeds that can survive for more than 20 years (Brown & Porter, 1942; Frazier, 1943b; 1948; Mitich, 1991; Schoenhals et al., 1990; Timmons, 1949). Due to these characteristics, field bindweed has been able to spread and occupy large expanses of the continental United States and Hawaii (USDA NRCS, 2013). This plant was brought to the United States as early as 1739 from Europe and Asia in contaminated crop seed (Weaver & Riley, 1982). By 1908, field bindweed was already recognized as a problem in cropping systems, infesting all of the western United States (Mitich, 1991; Weaver & Riley, 1982).

Although cultural, mechanical, and chemical control methods are useful for managing field bindweed in localized areas, land managers have utilized biological control methods to manage this plant over larger landscapes. *Aceria malherbae* Nuzzaci is an eriophyid mite that has been released to manage field bindweed in Texas (Boldt & Sobhian, 1993) and the Great Plains, including Montana (McClay et al., 1999). Establishment of *A. malherbae* in New Mexico and Texas has been successful with as much as 90% weed biomass reduction observed in field bindweed locations where the mite has established (Smith et al., 2010). *Aceria malherbae* induce galls on the leaves of field bindweed and these galls simultaneously provide food and protection for these mites. Between 1992 and 1995 populations of *A. malherbae* from Temple, Texas and Thessaloniki, Greece were introduced to sites in Montana (McClay et al., 1999). Establishment of *A. malherbae* in Montana was variable and subsequent dispersal was patchy (McClay et al., 1999). Smith et al. (2010) suggested that late freezes, snow, or hail after mite emergence from overwintering could be impacting the mites' ability to develop populations that are large enough to damage field bindweed's biomass and reproduction. However, climate may not be the only factor likely to affect the establishment of *A. malherbae*. Laurialt et al. (2010) also noted that management options, such as mowing could affect *A. malherbae* establishment.

Hypotheses and Objectives

Field bindweed sites were initially surveyed in 2007 to determine the status of *A. malherbae* establishment in Montana (Littlefield, 2007). The objective was to perform an exploratory analysis of similarities among sites based on the measured *A. malherbae* levels and to identify specific habitats conducive to mite establishment, with the hypothesis that sites with similar habitat characteristics would have comparable *A. malherbae* infestation levels.

In 2012 a portion of the 2007 sites were surveyed again to obtain a better understanding of the factors which affected within-field mite establishment; with the hypothesis that habitat characteristics influence intra-site mite establishment.

Materials and Methods

Sampling Design and Measurements

Sampling in 2007. The 2007 initial *A. malherbae* release sites across Montana (McClay et al., 1999) were visited during the summer of 2007 to determine the extent of establishment and mite dispersal from initial release sites. It was quickly determined that *A. malherbae* had widely dispersed from one or more of the sites where *A. malherbae* was released during the 1990s and 2000s (McClay et al., 1999); therefore, a wider and more general survey was conducted with a total of 71field sites visited (Figure 4-1; Appendix A). Field bindweed was present at all sites, and primarily located along major and secondary highways, recreational areas, and vacant land. At each site, location information and habitat characteristics were recorded. Location information included latitude, longitude (decimal degrees) and elevation (m). Habitat characteristics included field bindweed patch size (m^2), cover class categories, and a disturbance categorization. The cover class categories measured percent cover of grasses, forbs, bare ground, and field bindweed using the modified Braun-Blanquet method (Hill, 2005). The disturbance categorization was on a scale from 1-5 (1=undisturbed to 5= highly disturbed/cultivated). The level of mites was categorized and the mite density was quantified within the site area. Additionally, the gall levels at each site included none (0), low (1), medium (2), or high (3) gall infestation.

Sampling in 2012. In 2012, 15 sites were surveyed to measure habitat characteristics (e.g. cover classes of grass, forb, bare ground, and field bindweed) that differed within areas of the site where mites were present or absent (Figure 4-2; Appendix B). Nine of the 2007 survey sites having high mite infestations in 2007 were revisited as well as six newly selected field bindweed sites. The 2012 sites were chosen based on location, known field bindweed presence, and suspected *A. malherbae* presence. Selected sites had greater than 10 square meters of total bindweed infestation, although not necessarily in continuous or unbroken patches.

To determine mite presence or absence at a site, a meandering survey was conducted for a 30 minute period. The time until the first *A. malherbae* infested plant was observed was used as an indication of search effort. If no evidence of mites were found during the first 30 minutes, a site was considered absent of mites.

At sites where mites were present, a Daubenmire quadrat (0.125 m^2) (Daubenmire, 1959) was established where the mites were first observed. A separate quadrat, not infested with *A. malherbae*, was selected when possible within one meter of the galled field bindweed quadrat) and was used to compare differences between galled and uninfested field bindweed quadrats in the same field. A random direction was chosen and the next quadrats (one with galls present and one with galls absent) were established at least five meters from the previous. Mite abundance based on the number of galls per stem and the percent of stems infested by galls, and the percent cover of grass, forb, litter, and bare ground for each quadrat was recorded. Between three and ten quadrat pairs were used per field depending upon plant or mite density.

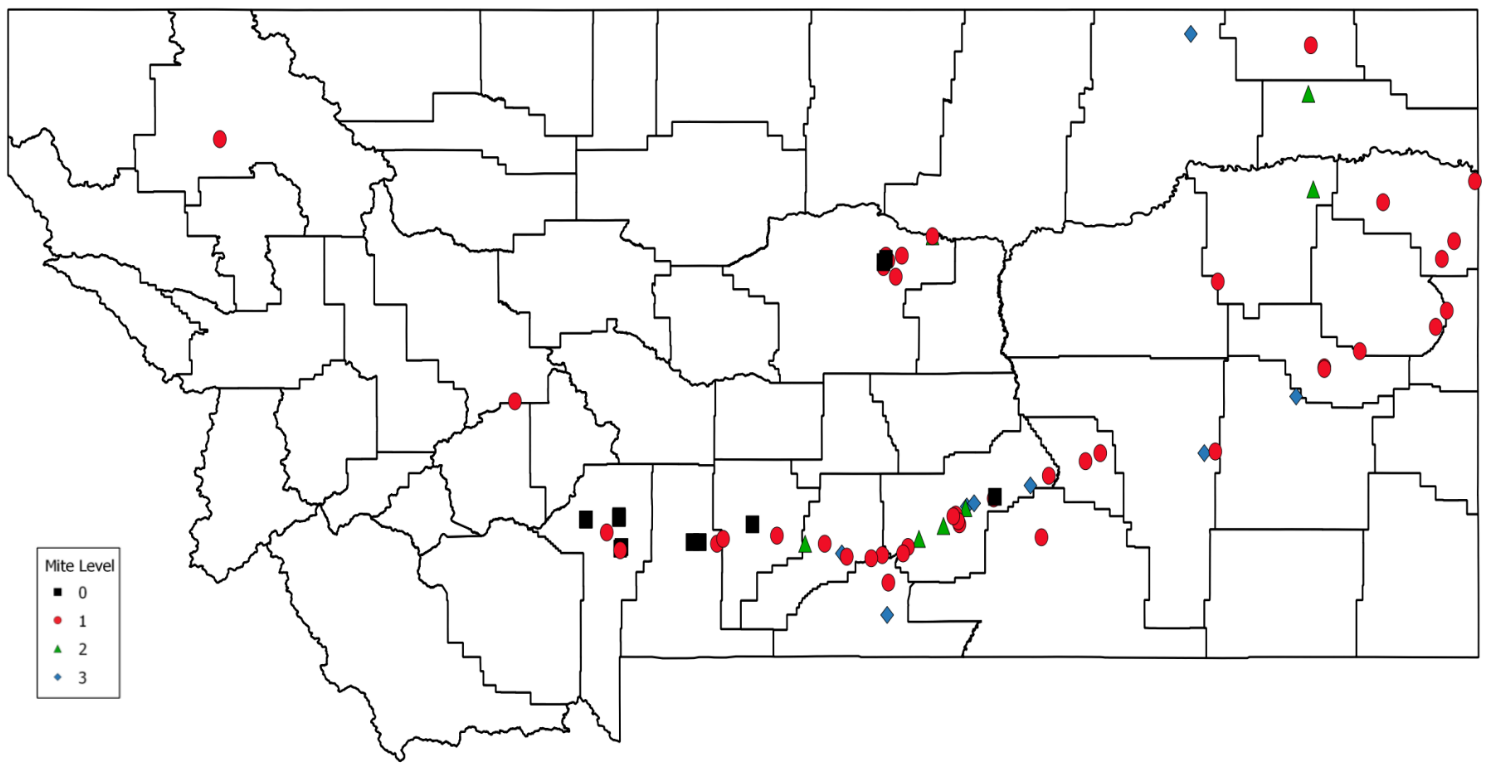


Figure 4-1. Map of Montana Showing 2007 Survey Sites Categorized by *Aceria Malherbae* Abundance

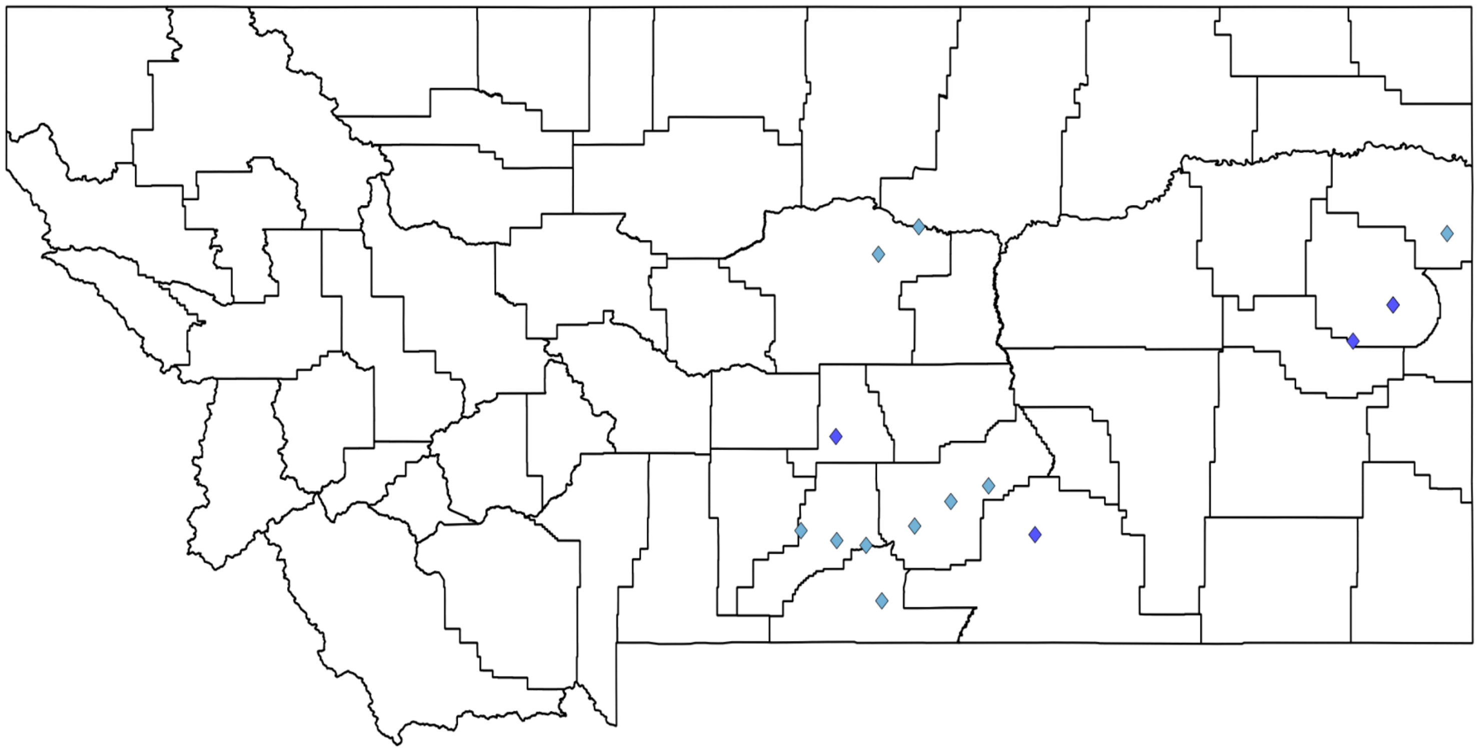


Figure 4-2. Map of Montana Showing 2012 Field Bindweed and *Aceria Malherbae* Survey Sites

Statistical Analysis

Analysis of 2007 Data. A Gower's distance matrix was constructed on all of the measured variables for the 2007 *A. malherbae* survey, and was analyzed using multidimensional scaling (MDS) (Maechler et al., 2012). Gower's Distance Similarity Index is particularly useful for mixed datasets consisting of ordered, categorical, and numerical variables (Becue-Bertaut & Pages, 2008; Gower, 1971) to visualize the high dimensional data on a lower dimensional scale. Multidimensional scaling was used to visualize patterns in the data and to investigate whether site variables were related to the success or failure of *A. malherbae*.

Permutation analysis of variance (PERMANOVA) (Anderson, 2001; Oksanen et al., 2013) was subsequently used to determine if the resulting similarity matrices of combined original variables corresponded to the known categorical mite levels using the vegan package in R (Oksanen et al., 2013). Full and subsets of the habitat and location variables were used as the explanatory variables in the PERMANOVAs. Habitat variables included patch size, habitat category, plant cover, and disturbance; location variables included latitude, longitude, and elevation; and a third matrix of similarities combined these two subsets of variables. The gall count category was used as the response variable using one thousand permutations for the PERMANOVA (Anderson, 2001). The PERMANOVA tested the null hypothesis of no differences in habitat matrix variables, location variables, and a combined matrix differed among mite levels.

Analysis of 2012 Data. Multiple logistic regressions using generalized linear models in R were used for the 2012 data (R Development Core Team, 2011). These analyses used non-Gaussian distributions based on the characteristics of the data collected. The analysis of counts of stems galled used the binomial analysis to model the odds of a stem being galled. The associated characteristics of this variable were binary responses (number of stems galled, number of stems not galled). The Poisson analysis was used to model the concentration of galls in quadrats. Poisson distributions are typically used for count data, so the number of galls on each bindweed stem fit this distribution. Finally, the binary analysis was used to compare differences of quadrats that contained galled plants to quadrats where none of the plants were galled. Since the response variable being analyzed was presence or absence within a quadrat, the binary distribution fit the dataset. All of these analyses used the log link function for the response variable and grass, forb, bare, and bindweed cover as the explanatory variables to base the models on. These analyses also accounted for the lack of independence between quadrats by including site as an explanatory variable.

When the variance found in the modeled data did not meet the criteria of a Poisson or binomial distribution (based on the mean variance association expected by the distribution in question), a quasi-likelihood correction was used to correct for the over dispersion detected (Richards & Shane, 2008). To do this, a variance inflation factor was calculated using the Pearson deviance dispersion parameter in R (R Development Core Team, 2011). This process was used for the binomial analysis of counts per stems galled and the Poisson analysis of concentration of galls within a quadrat.

Results

Results of 2007 Analysis

The variables used in the multidimensional scaling analyses and associated PERMANOVA are summarized in Table 4-1. Averages and standard deviations of quantitative location variables by mite level are presented in Table 4-2. A summary of the most common categorical variables are in Table 4-3. These summaries describe the range of sites visited during the 2007 survey.

Table 4-1. Summaries of Quantitative Location Variables and Categorical/Ordinal Habitat Variables Combined into a Single Gower Distance Matrix and Analyzed Separately

Variable	Units and Levels	Description
Latitude	decimal degrees	45.26 - 48.85°
Longitude	decimal degrees	104.07 - 114.33°
Elevation	Meters	607 – 1478 m
Patch Size	meters squared	10 - 22500 m^2
Habitat	8 categories	>50 % of observations are "roadside"
Cover Class	6 unequal ordinal cover categories	grass, forb, bare, and bindweed percent cover
Disturbance	5 ordinal categories	1=undisturbed to 5= highly disturbed/cultivated

Table 4-2. Quantitative Variables[1] Measured in 2007 Survey Summarized by *Aceria Malherbae* Level

Mite Level (N sites)	Longitude (°)	Latitude (°)	Elevation (m)	Patch Size (m^2)
0 (14)	110.40 (± 1.07)	46.04 (± 0.61)	4231.71 (± 636.15)	341.07 (± 941.02)
1 (42)	107.94 (± 2.30)	46.59 (± 0.90)	3093.83 (± 669.36)	1077.02 (± 3804.05)
2 (7)	107.73 (± 1.64)	46.74 (± 1.21)	2899.86 (± 549.39)	2392.86 (± 3779.60)
3 (8)	107.55 (± 1.34)	46.32 (± 1.10)	3065.50 (± 474.43)	415.00 (± 857.43)

[1]Each value is the mean of N number of sites (± SD)

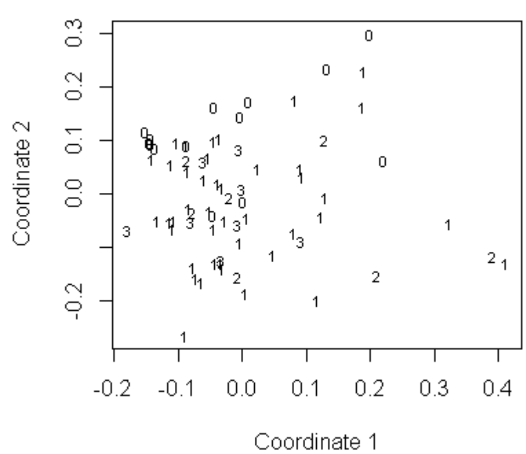
Table 4-3. Categorical Variables in Each Category Measured by *Aceria Malherbae* Level

Mite Level[1] (N sites)	Habitat	Field Bindweed (Cover Class*)	Grass (Cover Class)	Forb (Cover Class)	Bare Ground (Cover Class)	Disturbance (Class**)
0 (14)	Roadside (9)	1 (11)	3 (9)	1 (9)	2 (6)	3 (7)
1 (42)	Roadside (23)	1 (22)	3 (20)	1 (23)	2 (18)	3 (22)
2 (7)	Roadside (3)	2 (5)	3 (5)	1 (4)	3 (3)	3 (3)
3 (8)	Roadside (5)	2 (3)	2.5*** (4)	1 (5)	2.5*** (3)	3 (7)

[1] Nnumber of sites for each variable
Total count in each *Aceria malherbae* category indicated in the first column
*Cover Class: 1=0-5% cover, 2=2-25%, 3=25-50%, 4=50-75%, 5=75-95%, 6=95-100%
**Disturbances ranges from 0=undisturbed to 5=highly disturbed/cultivated
***Mean of 2 medians/modes

The percent variation in the full matrix of location and habitat variables explained by the two dimension approximation of the calculated distance matrix was 38% (Figure 4-3). These possible groupings among the four gall level classifications (tested with PERMANOVA to detect differences in gall level categories) resulted in a significant p-value, suggesting that the similarity between sites and the gall counts were related ($F=2.78$, $R^2= 0.11$, $P= 0.003$, $DF=3$).

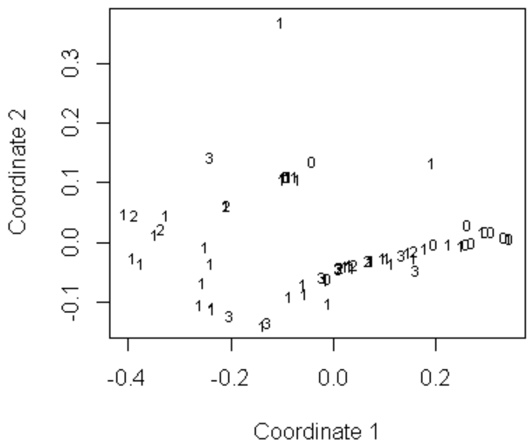
To further identify characteristics associated with mite establishment, the Gower distance matrix of the location variables including elevation, latitude and longitude were visualized (Figure 4-4) and also subjected to PERMANOVA. The PERMANOVA of the Gower's distance matrix of latitude, longitude, and elevation was significant (F=6.96, R^2=0.24, P=0.001, DF=3), indicating that there was a spatial relationship with *A. malherbae* establishment. The multidimensional scaling plot of the habitat distances, which included factors such as patch size, habitat category, plant cover, and disturbance (Figure 4-5) indicated no obvious pattern of clustering based on gall count, and the associated PERMANOVA of the Gower's habitat distances relationship with mite level was not significant (F=1.37, R^2=0.06, P=0.203, DF=3).



Symbols signify gall level:
0: No Galls To 3: High Gall Level

38% of the variation explained by the two dimensions

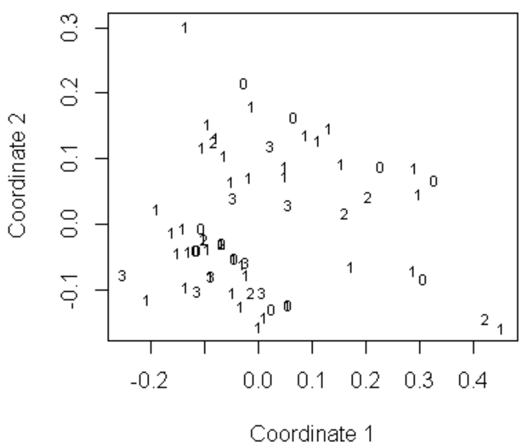
Figure 4-3. Classical Multidimensional Scaling Visualization of the Gower's Distance of 2007 Site Similarity for Combined Location and Habitat Data



Symbols signify gall level:
0: No Galls To 3: High Gall Level

40% of the variation explained by the two dimensions

Figure 4-4. Classical Multidimensional Scaling Visualization of the Gower's Distance of 2007 Site Similarity for Location Data (Latitude, Longitude, and Elevation).



Symbols signify gall level:
0: No Galls To 3: High Gall Level

40% of the variation explained by the two dimensions

Figure 4-5. Classical Multidimensional Scaling Visualization of the Gower's Distance of 2007 Site Similarity for Habitat Data (Patch Size, Grass Cover Class, Forb Cover Class, Bare Cover Class, Field Bindweed Cover Class, and Disturbance Category).

<u>Results of 2012 Analyses</u>

Seventy two vegetation quadrats from 15 sites (Table 4-4) were included in the Poisson logistic regression of the count of plants galled (odds of being galled) and binomial logistic regression of the density of galls per stem. In the binomial analysis, no difference was found for the cover estimates between quadrats in sites surveyed (Table 4-4). In the Poisson analysis, no difference was found for most of the cover estimates in the log of the odds of the stems being galled, however, grass cover was found to significantly affect gall numbers. In general galls increased with grass percent cover (T=2.302, P=0.025) with an estimated 1.01 times increase for every one percent increase in grass cover. This difference is not likely to be biologically significant since the increase is so small as to likely be negligible.

A separate binary logistic regression of presence or absence of galls within a quadrat was performed on the same 72 galled quadrats in addition to 57 quadrats where no galls were observed (Table 4-5). A total of 129 quadrats were analyzed for gall presence. This analysis indicated the odds of being galled were 1.06 times more likely for every one percent increase in field bindweed cover. This association could be due to the non-random sampling of non-galled quadrats as a result of the criteria imposed by the sampling plan since only the quadrats with minimal field bindweed presence could have a possibility of having no gall numbers in heavily gall infested areas.

Table 4-4. Percent Cover[1] for 15 *Aceria Malherbae* Infested Sites Sampled in 2012

GPS Location	Quadrats (no.)	County	Grass Cover (%)	Forb Cover (%)	Litter Cover (%)	Bare Ground (%)	Field Bindweed Cover (%)	Galled Stems (no.)	Ungalled Bindweed Stems (no.)	Galls (no.)	Bindweed Stems (no.)
46.90°N 105.02°W	5	Dawson	40.00 (±9.08)	2.00 (±2.00)	26.00 (±8.12)	18.00 (±13.56)	14.00 (±2.92)	14.60 (±4.76)	2.20 (±1.11)	123.80 (±42.00)	16.80 (±5.07)
45.73°N 108.61°W	5	Yellowstone	1.20 (±1.20)	16.20 (±6.36)	12.20 (±3.51)	9.00 (±4.58)	61.20 (±9.79)	22.80 (±11.36)	31.00 (±18.73)	204.80 (±102.05)	53.80 (±17.83)
45.26°N 108.88°W	5	Carbon	37.00 (±14.46)	9.00 (±7.81)	41.00 (±16.23)	8.00 (±8.00)	5.00 (±0.00)	3.40 (±1.69)	1.60 (±1.17)	13.60 (±6.70)	5.00 (±2.77)
47.62°N 108.58°W	5	Fergus	35.00 (±6.71)	22.00 (±3.39)	9.00 (±2.92)	25.00 (±7.42)	9.00 (±2.92)	2.80 (±0.66)	3.40 (±1.21)	8.00 (±2.07)	6.20 (±1.77)
45.64°N 109.25°W	3	Stillwater	24.33 (±7.22)	1.67 (±1.67)	36.67 (±25.22)	9.33 (±6.98)	27.67 (±11.57)	15.67 (±3.84)	2.33 (±1.20)	171.00 (±84.11)	18.00 (±4.00)
47.57°N 104.25°W	3	Richland	76.67 (±10.93)	0.00 (±0.00)	18.33 (±10.93)	0.00 (±0.00)	5.00 (±0.00)	1.67 (±0.67)	0.67 (±0.67)	24.00 (±22.01)	2.33 (±0.67)
47.13°N 104.69°W	5	Dawson	47.00 (±10.32)	4.00 (±1.87)	17.00 (±5.39)	10.00 (±5.24)	22.00 (±3.74)	7.40 (±1.69)	4.20 (±2.58)	83.20 (±23.71)	11.60 (±2.01)
47.13°N 104.69°W	10	Dawson	55.40 (±8.28)	4.30 (±1.50)	16.40 (±2.35)	11.90 (±6.60)	11.90 (±3.12)	6.00 (±2.92)	3.10 (±1.07)	23.30 (±13.02)	9.10 (±2.78)
45.68°N 107.62°W	5	Big Horn	11.00 (±3.67)	8.00 (±8.00)	25.00 (±12.65)	18.00 (±8.15)	38.00 (±11.58)	3.80 (±2.56)	22.20 (±9.14)	71.00 (±69.00)	26.00 (±7.71)
45.89°N 108.31°W	5	Yellowstone	35.00 (±5.92)	0.00 (±0.00)	54.00 (±7.97)	0.00 (±0.00)	11.00 (±2.92)	6.40 (±2.93)	7.80 (±2.01)	40.00 (±26.72)	14.20 (±2.42)
47.46°N 108.87°W	5	Fergus	33.00 (±8.31)	5.00 (±3.16)	33.00 (±6.63)	24.00 (±9.14)	5.00 (±0.00)	1.40 (±0.24)	3.80 (±0.97)	3.20 (±0.73)	5.20 (±0.97)
45.99°N 108.00°W	3	Yellowstone	73.33 (±3.33)	3.33 (±3.33)	15.00 (±5.00)	0.00 (±0.00)	8.33 (±1.67)	1.67 (±0.33)	2.00 (±1.00)	9.00 (±4.16)	3.67 (±0.88)
45.71°N 109.54°W	5	Sweetgrass	8.00 (±3.39)	2.00 (±1.22)	44.00 (±12.59)	1.00 (±1.00)	45.00 (±13.42)	19.60 (±5.30)	14.80 (±8.49)	125.80 (±54.93)	34.40 (±11.48)
46.30°N 109.26°W	4	Golden Valley	61.00 (±10.17)	8.50 (±5.68)	4.75 (±2.06)	3.50 (±1.19)	22.25 (±9.71)	40.00 (±13.55)	6.00 (±2.04)	125.50 (±50.60)	46.00 (±12.12)
46.61°N 109.01°W	5	Stillwater	21.80 (±13.63)	8.80 (±5.61)	46.80 (±13.28)	2.00 (±1.22)	20.80 (±6.22)	9.00 (±2.74)	11.60 (±5.10)	96.80 (±60.29)	20.60 (±3.97)

Each value is the mean of the number of quadrats per site (± SE)

Table 4-5. Percent Cover[1] by *Aceria Malherbae* presence of *A. malherbae* Infested Sites Sampled in 2012

Galls	Grass (%)	Forb (%)	Litter (%)	Bare Ground (%)	Field Bindweed (%)
Absent	39.56 (±3.48)	6.33 (±1.44)	33.25 (±3.39)	10.53 (±2.30)	10.33 (±1.55)
Present	36.52 (±3.22)	6.53 (±1.21)	26.48 (±2.77)	10.08 (±1.91)	20.36 (±2.44)

[1] Each value is the mean of 73 galled (+) and 57 ungalled (-) quadrats (±SE)

Discussion

Habitat characteristics surveyed were largely found to not be associated with mite levels. Based on the analyses of the 2007 survey, a closer examination of characteristics associated with latitude, longitude and elevation, such as climatic differences of locations may be needed to further refine relationships between gall abundance and favorable sites. Sampling bias based on the proximity to initially established sites may also play a role in the relationship between location and mite level. These characteristics associated with the presence or absence of *A. malherbae* can be further refined and investigated in future studies.

Though there was a small increase in gall count found associated with the probability of stems being galled, it is unclear why these higher gall associations were found in the Poisson log linear regression analysis. One possible explanation was that grasses had a growing pattern with more open ground between grass patches compared with forbs that could create environmental conditions that were more favorable for increased gall induction. However, open ground was not directly related to the gall counts in analyses. Perhaps the grass cover had an association with other conditions that were favorable to gall induction or conditions which minimized mite dispersal such as the ability to move from plant to plant within a quadrat. This possibility might explain why the odds of the galls being present did not increase with an increase in grass percent, but the count of the galls did increase. The 1.01 times increase for every one percent increase in grass cover is likely not biologically significant, however.

Overall, no clear evidence that habitat characteristics such as percent cover of the plant groups affected *A. malherbae* establishment was found in the 2007 and 2012 studies. Mite establishment varied among and within sites due to other unknown factors. Further research could be used to identify what it is that causes the patchy establishment. For example, habitat characteristics that promote transfer from site to site, such as wind speed and direction and the development of a quantification process to measure distance and time between patches surveyed might prove to be useful avenues of research to determine where mites will best establish following establishment at a given site.

Observations of differences in mite levels between the 2007 and 2012 surveys could be used to develop hypotheses on mite establishment causes. For example, temporal variables at sites might have an effect on mite population. Of particular note was a site in Vida, Montana visited in 2007 was found to have high mite populations but was absent of *A. malherbae* in 2012. One key observation was that the field was highly disturbed, having been recently tilled. This heavy disturbance would be a strong justification for the change in the mite levels since the habitat where the mites can continuously reproduce was damaged, and only newly growing bindweed was observed for mite presence. Similarly, weather patterns could affect mite establishment. This was seen in 2011 and 2012 visits to an *A. malherbae* collection site in Huntley, Montana.

Where mites had been abundant historically, including 2007 an many years after, populations were sparse in 2011 following a particularly wet year, yet were observed to rebound in 2012. As Ulrichs and Hopper (2008) pointed out, of the number of climate and habitat factors available to map species distribution on a large scale, no single factor was seen to affect all the six insect pest species they examined. Instead, a unique combination of predictor variables was identified for each species (Ulrichs & Hopper, 2008). Further observational and experimental studies could determine what site characteristics have the largest effect on mite population. In this study, only minimal impacts of habitat on mite populations were observed. However, the variables tested were not a conclusive list of habitat characteristics. Investigating other relationships between mite abundance and habitat characteristics might be warranted to better understand factors affecting *A. malherbae* and future similar biological control agent establishment.

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APPENDICES

APPENDIX A

SITE CHARACTERISTICS OF 71 SITES SURVEYED IN 2007

Appendix A: Site characteristics of 71 sites surveyed in 2007[1]

Latitude and Longitude	County	Gall*	Elevation (m)	Field Bindweed (Stems per 0.25 m)	Gall (no.)	Mite Level	Habitat	Bindweed Cover	Grass Cover	Forb Cover	Ground Cover	Slope	Disturbance **	Patch Size
47.48°N 108.75°W	Fergus	P	938	12	1	1	Hay	2	3	0	3	5	3	100
45.77°N 111.17°W	Gallatin	P	1369	10	1	1	Vacant	2	2	2	2	0	4	40
45.83°N 109.97°W	Sweetgrass	A	1230	15	0	0	Vacant	0	3	1	4	0	3	150
45.73°N 108.61°W	Yellowstone	P	996	21	14	2	Vacant	3	2	3	3	0	4	500
47.36°N 108.80°W	Fergus	P	989	8	1	1	Roadside	1	3	0	2	0	3	30
45.69°N 111.04°W	Gallatin	A	1456	8	0	0	Vacant	1	1	2	4	0	5	60
45.69°N 111.04°W	Gallatin	A	1456	6	0	0	Roadside	1	2	3	2	0	4	250
45.26°N 108.88°W	Carbon	P	1141	20	18	3	Tree farm	2	3	0	3	0	3	2500
45.67°N 111.06°W	Gallatin	A	1478	10	0	0	Roadside	2	3	1	2	0	4	250
45.67°N 111.06°W	Gallatin	P	1475	6	1	1	Roadside	2	3	1	2	0	4	200
47.61°N 108.50°W	Phillips	P	699	15	6	2	Native	2	3	1	2	0	2	600
47.61°N 108.50°W	Phillips	P	693	8	1	1	Native	1	2	1	4	0	2	120
45.64°N 109.25°W	Stillwater	P	1099	25	23	3	Vacant	3	2	1	2	0	3	20

47.57°N 104.25°W	Richland	P	607	3	1	1	Hay	1	4	2	2	0	4	100
46.13°N 107.56°W	Yellowstone	P	835	38	8	1	Roadside	3	2	2	3	0	3	2000
46.62°N 105.54°W	Custer	P	721	23	23	3	Roadside	3	2	1	2	20	3	100
48.85°N 106.39°W	Valley	P	991	7	7	3	Pasture	2	3	1	3	0	4	25
46.90°N 105.02°W	Dawson	P	686	16	1	1	Roadside	3	3	1	2	0	3	150
45.70°N 110.26°W	Park	P	1312	6	3	1	Roadside	1	3	1	3	10	3	25
46.26°N 106.28°W	Rosebud	P	771	20	20	3	Roadside	1	3	2	3	3	3	25
45.46°N 108.86°W	Carbon	P	1063	8	1	1	Roadside	1	3	1	3	0	3	100
45.82°N 108.41°W	Yellowstone	P	955	24	6	2	Roadside	2	3	2	3	0	3	5000
45.63°N 109.20°W	Stillwater	P	1160	6	1	1	Cropland	1	2	2	4	5	5	50
47.14°N 104.31°W	Wibaux	P	866	10	1	1	Roadside	1	4	2	2	35	3	50
45.68°N 108.71°W	Yellowstone	P	1000	22	3	1	Roadside	2	3	2	3	0	3	300
45.71°N 110.40°W	Park	A	1329	6	0	0	Roadside	1	3	0	3	5	3	80
45.72°N 110.47°W	Park	A	1348	3	0	0	Roadside	1	3	1	2	0	3	50
46.59°N 111.92°W	Lewis & Clark	P	1299	5	1	1	Vacant	1	1	1	5	0	5	10
47.33°N 106.17°W	Garfield	P	752	19	2	1	Roadside	3	2	1	3	0	4	100

45.76°N 109.77°W	Sweetgrass	P	1186	8	1	1	Pasture	1	3	1	3	0	3	150
45.74°N 107.61°W	Big Horn	P	870	4	1	1	Vacant	2	3	2	3	0	3	22500
46.28°N 106.20°W	Rosebud	P	758	16	2	1	Vacant	2	3	2	2	0	4	50
45.93°N 108.23°W	Yellowstone	P	917	29	29	3	Roadside	4	3	0	1	0	3	500
45.93°N 108.24°W	Yellowstone	P	924	14	11	2	Roadside	2	3	1	2	0	3	300
45.89°N 108.31°W	Yellowstone	P	934	25	13	1	Hay	3	2	2	3	0	4	5000
48.21°N 114.33°W	Flathead	P	908	5	1	1	Vacant	1	3	1	3	0	4	60
45.86°N 111.33°W	Gallatin	A	1298	9	0	0	Roadside	1	2	1	4	0	4	100
45.86°N 111.33°W	Gallatin	A	1301	5	0	0	Vacant	1	2	1	4	0	5	50
47.48°N 108.89°W	Fergus	P	937	6	1	1	Roadside	1	3	0	2	5	3	20
47.47°N 108.89°W	Fergus	A	945	6	0	0	Roadside	1	3	1	3	0	3	25
47.41°N 108.91°W	Fergus	P	973	5	1	1	Roadside	1	3	1	3	0	3	25
45.99°N 108.00°W	Yellowstone	P	883	3	1	1	Roadside	1	4	1	2	0	2	150
45.99°N 108.00°W	Yellowstone	A	885	2	0	0	Cropland	1	2	2	4	0	5	3600
45.64°N 108.92°W	Stillwater	P	1036	25	10	1	Roadside	3	2	1	3	0	3	150
45.71°N 109.54°W	Sweetgrass	P	1146	10	5	2	Roadside	1	3	1	3	5	3	50

47.44°N 108.91°W	Fergus	A	1060	20	0	0	Vacant	2	3	1	2	0	2	60
47.46°N 104.34°W	Richland	P	607	32	1	1	Roadside	4	4	0	1	0	4	75
48.78°N 105.42°W	Daniels	P	754	13	1	1	Vacant	2	2	2	4	0	5	25
45.82°N 108.29°W	Yellowstone	P	963	19	4	1	Roadside	2	2	0	4	0	3	400
45.85°N 108.30°W	Yellowstone	P	954	11	1	1	Roadside	2	3	1	2	5	3	150
45.64°N 108.75°W	Yellowstone	P	998	10	2	1	Vacant	1	4	0	1	0	2	50
45.74°N 110.21°W	Park	P	1269	15	1	1	Roadside	3	3	0	3	0	3	2500
45.87°N 111.06°W	Gallatin	A	1446	3	0	0	Roadside	1	3	1	2	0	3	25
45.87°N 111.06°W	Gallatin	A	1415	5	0	0	Roadside	1	3	1	2	0	3	25
45.86°N 111.06°W	Gallatin	A	1409	8	0	0	Roadside	1	3	0	3	0	3	50
46.79°N 105.30°W	Prairie	P	702	19	1	1	Roadside	3	2	1	2	20	3	50
46.78°N 105.30°W	Prairie	P	696	5	2	1	Hay	2	3	1	2	0	2	50
47.90°N 105.39°W	McCone	P	737	19	11	2	Cropland	2	1	2	5	5	5	10000
48.49°N 105.43°W	Roosevelt	P	736	10	4	2	Hay	2	3	1	4	5	5	300
45.96°N 108.16°W	Yellowstone	P	930	5	5	3	Roadside	2	2	1	4	0	3	100
45.70°N 109.39°W	Stillwater	P	1130	4	1	1	Vacant	1	3	1	2	0	3	20

46.06°N 107.71°W	Yellowstone	P	905	24	24	3	Roadside	4	2	1	2	30	3	50
45.87°N 108.33°W	Yellowstone	P	955	5	1	1	Roadside	1	2	1	4	0	4	20
46.22°N 107.25°W	Treasure	P	882	3	2	1	Roadside	1	4	0	3	5	3	25
47.94°N 104.07°W	Wibaux	P	861	5	1	1	Roadside	1	4	1	2	20	3	25
46.26°N 107.14°W	Rosebud	P	822	16	2	1	Hay	3	3	1	2	10	2	100
47.82°N 104.82°W	Richland	P	742	19	3	1	Cropland	2	0	2	5	5	5	10000
45.61°N 109.01°W	Stillwater	P	1046	12	2	1	Roadside	1	2	1	4	0	2	15
47.05°N 104.39°W	Dawson	P	797	5	1	1	Roadside	1	4	1	2	20	3	100

[1]Cover Class categories range from 1:0-5% cover to 6: 95 to 100%
*P indicates gall presence and A indicates gall absence
**Disturbance categories range from 1 not disturbed to 5 highly disturbed/cultivated

APPENDIX B

SITE CHARACTERISTICS OF 15 SITES SURVEYED IN 2012

Appendix B: Site characteristics of 15 sites surveyed in 2012

Latitude and Longitude (°)	Elevation (m)	Shape Category	First Gall Found (seconds)	Phenology	Visited in 2007	Grazing	Wild Animal	Cultivation	Herbicide	Mowing	Roadside	Recreation
46.90°N 105.02°W	681	Single Patch	109	Vegetative	N	-	-	-	-	-	-	+
45.73°N 108.61°W	977	Multiple Small Patches	120	Vegetative	Y	-	+ *	-	+	-	+	+
45.26°N 108.88°W	1148	Multiple Small Patches	38	Vegetative	Y	-	-	+	-	-	-	-
47.62°N 108.58°W	691	Linear	300	Vegetative	Y	-	-	-	-	-	+	-
45.64°N 109.25°W	1107	Single Patch	15	Immature Fruit	Y	-	-	-	-	-	+	-
47.57°N 104.25°W	594	Multiple Small Patches	95	Vegetative	Y	-	-	-	-	+	-	-
47.13°N 104.69°W	649	Single Patch	75	Vegetative	N	-	-	-	-	+	-	+
47.13°N 104.69°W	623	Single Patch	10	Vegetative	N	-	-	-	-	+	+	-
45.68°N 107.62°W	895	Linear	223	Mature Fruit	N	-	-	-	-	+	+	-
45.89°N 108.31°W	913	Single Patch	174	Vegetative	Y	-	-	-	-	+	+	-
45.99°N 108.00°W	1105	Linear	134	Mature Fruit	Y	-	-	-	-	+	+	+

45.71°N 109.54°W	1141	Single Patch	180	Vegetative	Y	-	-	-	-	-	+	-
47.44°N 108.91°W	951	Single Patch	896	Vegetative	Y	+	-	+	-	+	-	-
46.30°N 109.26°W	1120	Single Patch	100	Vegetative	N	-	-	-	-	+	+	+
45.61°N 109.01°W	1052	Linear	78	Vegetative	Y	-	-	-	-	+	+	-

*Ground squirrel holes present within quadrats