THESIS

INDIRECT INTERACTIONS BETWEEN GALLING INSECTS AS MEDIATED BY A NON-NATIVE PLANT

Submitted by

Hayley Lucci-Rimer

Graduate Degree Program in Ecology

In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Fall 2021

Master's Committee:
Advisor: Paul Ode
Co-Advisor: Meena Balgopal

Dan Bean
Troy Ocheltree

ABSTRACT

INDIRECT INTERACTIONS BETWEEN GALLING INSECTS AS MEDIATED BY A NON-NATIVE PLANT

Russian knapweed (*Rhaponticum* (=*Acroptilon*) *repens*) is a non-native plant species that has spread throughout the western United States, out-competing native and crop species. Two host-specific gall forming insects - the stem-gall wasp *Aulacidea acroptilonica* (Hymenoptera: Cynipidae) and the tip-gall midge *Jaapiella ivannikovi* (Diptera: Cecidomyiidae) - have been employed as biological control agents to manage its spread. However, the nature of the intra- and interspecific interactions between these two insect species is not fully understood even though this information is vital to controlling Russian knapweed. To determine the nature of these interactions (e.g., positive, negative, or neutral) and the effects that the insects have on Russian knapweed, I performed studies in the greenhouse and at field sites throughout Colorado. I found evidence for a negative or neutral interspecific relationship, a negative or neutral intraspecific relationship among midges, and a positive or neutral relationship among wasps. The nature of these interactions is likely density and resource dependent. The associations between each insect species and measures of plant growth and reproductive capacity were mixed across the two studies, at times showing positive, negative, or neutral relationships. More studies that consider insect density, water availability, and resource allocation within Russian knapweed should be performed to better determine how the insects interact with one another and what their effects on Russian knapweed may be under varying conditions.

ACKNOWLEDGEMENTS

I am very grateful for all the people and institutions that made this research possible. Paul Ode and Meena Balgopal offered tremendous support and mentorship throughout my time at Colorado State University. Dan Bean and Sonya Daly at the Palisade Insectary (Colorado Department of Agriculture) were essential in supplying insects, connecting me with land managers, and considering research possibilities. Troy Ocheltree provided feedback on my thesis and assistance with understanding plant ecophysiology. The Ode and Balgopal labs were helpful at several stages of my project, especially Theresa Barosh who offered insight into the study system, guidance in designing my research projects, and friendship. I was fortunate to work with excellent research assistants, Beatrice Lincke and Jamie Barosh, who were essential in making research ideas become realities. The Graduate Degree Program in Ecology, the Department of Agricultural Biology and the Department of Biology assisted me in numerous ways throughout my Master's program. Financial support came from the Agriculture and Food Research Initiative of the National Institute of Food and Agriculture (USDA). I am also grateful for the assistance of several land managers and owners who provided access to field sites, including Barbara Kruse, Suzanne Beauchaine at the Alamosa Wildlife Refuge, Kelly Uhing at Denver Parks and Recreation, Uriel Akiva at Commerce City Parks and Recreation, Glen Nishimoto at Xcel Energy, Josh Docheff at Diamond D. Dairy, and Steve Copley and Jinger Tilden at the Alamosa County Land Use office. I am deeply thankful for the love, kindness and patience of my wife Elizabeth Lucci-Rimer, who encourages me in research and inspires me in life.

TABLE OF CONTENTS

Chapter 1 – INTRODUCTION

Indirect Plant-Mediated Interactions

Interspecific and intraspecific interactions can strongly influence community assembly (Damman, 1993; Denno et al., 1995; Kaplan & Denno, 2007; McNutt & Underwood, 2016). Among phytophagous insects, these interactions are thought to commonly occur indirectly (Denno et al., 1995; Kaplan & Denno, 2007). Indirect interactions consist of one species influencing another species through its effects on the abundance and/or quality of a third mediating species. In the case of phytophagous insects, this third mediating species can be a plant, predator, or parasitoid that itself directly interacts with the two herbivorous insect species (Poelman & Dicke, 2018). A meta-analysis of 243 studies of phytophagous insect interactions found indirect interactions occurring in almost 90% of observations, with changes in plant quality responsible for mediating interactions in over 60% of observations (Kaplan & Denno, 2007). Changes in plant quality include changes in host plant defenses (Walling, 2000; Bezemer et al., 2003; Kaplan et al., 2008; Wurst et al., 2008; Erb et al., 2009; Soler et al., 2009; Price et al., 2011; van Dam & Heil, 2011), nutritive content (Inbar et al., 1995; Denno et al., 2000; Johnson et al., 2009), and morphology (Strauss, 1997; Traw & Dawson, 2002; Hall et al., 2012). These changes can cause the plant to become increasingly susceptible or resistant to other herbivores, resulting in positive (i.e., facilitation or commensalism) (Masters & Brown, 1992; Bronstein, 1994; Masters, 1995; Wallin & Raffa, 2001; Kaplan & Denno, 2007; Ohgushi, 2008; Bronstein, 2009) or negative (i.e., competition or amensalism) (Bezemer et al., 2003; Kaplan & Denno, 2007; Anderson et al., 2011; Cunan, 2015; Barnes & Murphy, 2018; Castagneyrol et al., 2021) interactions between the herbivores. Though both positive and negative interactions are possible, competition has been found to occur in over three times as many instances as facilitation (Kaplan & Denno, 2007). Additionally, the nature of the interaction between species may shift, becoming more competitive as herbivore densities increase (Pineda et al., 2017).

Classical competition theory predicts that intraspecific competition should be stronger than interspecific competition since competition is expected to intensify when two organisms exhibit greater overlap in resource requirements and co-occur spatially and temporally (Levins, 1968; Diamond, 1978; Kaplan & Denno, 2007). However, indirect interactions between organisms can occur even with spatial and temporal separation and minimal niche overlap (Damman, 1993; Kaplan & Denno, 2007). Therefore, when considering the prevalence of indirect interactions among phytophagous insects, competition between conspecifics may not always be greater than between heterospecifics. A meta-analysis of phytophagous insect interactions found no significant differences in the strength of competitive interactions between heterospecifics and conspecifics (Kaplan & Denno, 2007). When direct interactions do occur however, they are generally expected to be stronger than indirect interactions since direct effects are less likely to be dampened by the organisms that mediate indirect interactions, especially in variable environments that can perturb the responses of the mediating organisms (Schoener, 1993; Wootton, 1994, 2002).

Gall-forming insects are well-suited for the examination of indirect plant-mediated interactions since these insects spend the vast majority of their life cycle within their galls and interactions between insects inhabiting different galls occur almost entirely through the plant host. Galls are swollen plant tissues induced by oviposition and subsequent larval feeding. Galls offer protection from predators and parasitoids as well as nourishment to the developing larvae. Galls can draw photosynthates towards them from the plant’s source tissues, functioning as metabolic sinks. As such, galls can compete with other sinks, such as fruits, flower buds, and meristematic shoot and root tips (Larson & Whitman, 1997), preventing the plant from allocating resources to its own growth and reproduction (Schultz et al., 2013). When multiple insects form more than one gall on the same plant, the interaction of multiple gall-sinks can result in plant-mediated interspecific and intraspecific interactions (Burstein et al., 1994; Inbar et al., 1995; Kaplan et al., 2011; Barosh, 2020).

Study System

Russian knapweed (*Rhaponticum* (=*Acroptilon*) *repens*) is a nonnative species that spread throughout the western United States after its unintentional introduction from Eurasia in the late 1800s by way of imported alfalfa seed for forage production (Jacobs & Denny, 2006). Russian knapweed is a perennial plant that forms large monotypic patches by reproducing clonally through rhizomatic growth that gives rise to new, clonal stems that are known as ramets (Morrison et al., 1995). It can also spread to distant areas by way of seed dispersal (Djamankulova et al., 2008; Gaskin & Littlefield, 2017), with US populations producing almost four times more seed than knapweed plants in their native range and growing nearly twice as densely (Callaway et al., 2012). Russian knapweed produces 50-500 seeds per ramet, which remain viable in the soil for two to three years (Beck, 2003). This nonnative species out-competes native and crop species by forming dense stands that can degrade rangelands, croplands, and riparian corridors (Watson, 1980; Duncan et al., 2004). It has been shown to reduce wheat yield by 28-75%, corn yield by 64-88%, and cause a potentially fatal neurodegenerative disorder in horses if consumed (Watson, 1980; Chang et al., 2012). The economic impact of three different species of knapweed, including Russian knapweed, on

Montana's economy was estimated at $42 million annually (Hirsch & Leitch, 1996). As a result of these deleterious effects, Russian knapweed is classified as a List B Species in the Colorado Noxious Weed Act, which dictates that its spread must be controlled to prevent new populations from establishing, making it a high management priority for the Colorado Department of Agriculture (CDA) and private landowners.

Since Russian knapweed typically grows in large stands on remote lands, chemical herbicides are often not an environmentally or economically favorable management option (DiTomaso, 2000; Djamankulova et al., 2008). To prevent the spread of this nonnative species, two host-specific gall-forming biological control agents from Russian knapweed's native range - the gall wasp, *Aulacidea acroptilonica* (Hymenoptera: Cynipidae), and the gall midge, *Jaapiella ivannikovi* (Diptera: Cecidomyiidae), - were approved for release in the US in 2008 and 2009 respectively (USDA APHIS, 2008, 2009). The gall midge and the gall wasp display different galling and life cycle characteristics. Midges have a 1:1 sex ratio and mate in the spring soon after emerging from galls as adults (Djamankulova et al., 2008). The adult females live for an average of 3.5 days, with a range of 2 to 7 days, and oviposit on the growing bud tips of young plants, forming a rosette gall of fused leaves and preventing flower formation (Djamankulova et al., 2008). Midges are multivoltine as females emerge and oviposit about every two to four weeks throughout the growing season, with approximately four generations per year (Djamankulova et al., 2008; Meyers et al., 2015). Wasps are univoltine with a female biased sex ratio of 9:1 (Djamankulova et al., 2008). The females emerge only once per year in the spring with their eggs completely developed and live for an average of 5 days, with a range of 2 to 9 days (Djamankulova et al., 2008). During this brief time, they oviposit in the stem tissues, forming galls.

There have been promising results for the abilities of both galling insects to impair Russian knapweed growth and reproductive capacity. In a field study performed in the native range of Uzbekistan, where there were high densities of both insects, midges reduced shoot length by 12%, above-ground biomass by 24%, and seed output by 92%, while wasps reduced shoot length by 21%, above-ground biomass by 25%, and seed output by 75% (Djamankulova et al., 2008). However, no significant effects on Russian knapweed were observed in the non-native range of Wyoming that had low densities of midges and no wasps (Meyers et al., 2015).

Biological Control Considerations

The management of nonnative species using multiple biological control agents is one circumstance that demands a thorough understanding of how species interact with one another for management to be successful. A meta-analysis of 74 cases of multiple enemies attacking a host plant determined that non-independent interactions were exhibited between the biological control agents in about 25% of these instances (Stephens et al., 2013). Despite these findings, few biological control studies address the possibility that agents might interact, especially when considering the potential of indirect interactions (Milbrath & Nechols, 2014). Common models of classical biological control assume a neutral or positive relationship between multiple biological control agents. The lottery model encourages the use of multiple agents to increase the likelihood of at least one agent being effective against the target pest (Myers, 1985). The cumulative stress hypothesis predicts that multiple agents will act additively or even synergistically, resulting in greater damage to the target pest (Harris, 1981, 1991). However, if the interaction between agents is negative, then employing a single agent could be more effective than using multiple competing agents, especially if the agent that is most damaging to the target pest is the inferior competitor (Ehler & Hall, 1982; Leveque et al., 1993; Woodburn, 1996; De Moraes et al., 1999).

Several meta-analyses have found that negative relationships among biological control agents and phytophagous insects are more common than positive or neutral relationships. In a review of 193 pair-wise species interactions among phytophagous insects generally, it was found that 76% of interactions indicated interspecific competition, 18% indicated an absence of competition, while only 6% indicated facilitation (Denno et al., 1995). The same study further found that competition is more likely among introduced species that often lack natural enemies and reach high densities as well as among sessile species that have limited means of switching host plants and evading competition (Denno et al., 1995). In a meta-analysis of 74 cases of multiple enemies attacking a host plant, 14-22% of interactions were antagonistic, particularly when agents established on the same host plant, attacked the same part of the plant, or attacked reproductive plant structures (Stephens et al., 2013). Furthermore, a single biological control agent has been found to be responsible for the suppression of a target weed in about 50-80% of cases where multiple species have been released (Myers et al., 1985; Myers et al., 1989; Denoth et al., 2002). Therefore, if the relationship between agents is negative, it may be more beneficial to release only the agent that is most successful at controlling the target species.

Studies

I performed two studies to determine the nature (i.e., positive, negative, or neutral) of the intra- and interspecific interactions between the midge and the wasp as well as to examine how each insect, together and separately, affects Russian knapweed growth and reproductive capacity. The study in the first chapter is a field study that was conducted at 16 field sites throughout

Colorado. Midges and wasps were released in a 2x2 factorial design with midge and wasp presence and absence. This part of the study focused on the interspecific interaction between midges and wasps and their impacts on Russian knapweed under realistic field conditions. The study described in the second chapter was similarly designed in a 2x2 factorial manner with midge and wasp presence and absence across 60 individually potted plants in a greenhouse. This portion of the study addressed the type of interaction occurring between gall midges and gall wasps at a ramet level in greater detail than in the field. It also allowed for a more thorough investigation of the physiological impacts of the galling insects on Russian knapweed in terms of photosynthetic rates and resource allocation. Both studies were performed in the spring and summer of 2019 in Colorado.

Chapter 2 – BIOLOGICAL CONTROL AGENTS EXHIBIT NEGATIVE INTERSPECIFIC INTERACTIONS WHEN ESTABLISHED ON SEPARATE RAMETS

INTRODUCTION

Most plants are attacked by multiple herbivore species, raising the question of how these species interact with one another. Such interactions may be negative (-), positive (+), or neutral (0) (Moon et al. 2010). Within a trophic level, these relationships can result in competition (-/-) (Karban, 1989; Leveque et al., 1993; Denno et al., 1995; Woodburn, 1996; Redman & Scriber, 2000; Reitz & Trumble, 2002), facilitation or mutualism (+/+) (Denno & Kaplan, 2007; Marquis & Lill, 2007; Johnson et al., 2009), amensalism (0/-) (Wetzel et al., 2016), commensalism (0/+) (Lill & Marquis, 2003; Vieira & Romero, 2013), or neutralism (0/0) (Moon et al., 2010; Mouttet et al., 2011). The outcome of these interspecific relationships not only determines how herbivores interact with each other but also how they interact with their host plants, with the potential for plant traits to mediate herbivore interactions (Stephens et al., 2013).

Interspecific relationships have traditionally and most frequently been studied through the lens of direct interactions, wherein organisms physically interact with one another at the same time and place (Damman, 1993; Denno et al., 1995; Denno & Kaplan, 2007; Kaplan & Denno, 2007). However, indirect interactions, wherein two organisms influence one another through a third mediating organism of a different species and typically in a different trophic level, are perhaps the most common form of interaction among herbivorous insects and can occur even if the insects' attacks are spatially or temporally separated (Damman 1993; Denno et al., 1995; Denno & Kaplan, 2007; Kaplan & Denno, 2007; Anderson et al., 2011; Stephens et al., 2013). Despite the prevalence of these interactions, few studies address the possibility that biological control agents might interact, especially when considering the potential of indirect interactions (Stephens et al., 2013; Milbrath & Nechols, 2014). However, it is essential to understand how agents interact with one another to predict the outcome of biological control efforts (Stephens et al., 2013). This study addresses the possibility of interaction between two herbivorous biological control agents and discusses the implications of this interaction for success in controlling their nonnative plant host.

Classical biological strategies using multiple agents assume a positive or neutral relationship (i.e., facilitation, commensalism, or neutralism) between agents. For example, the lottery model predicts that releasing multiple agents will increase the likelihood of the suppression of the target pest since there is a greater chance of one agent having powerful negative effects on the pest (Myers, 1985). The cumulative stress hypothesis predicts that the effects of the multiple agents will be additive or even synergistic, with more agents causing greater damage to a target pest, leading to better control (Harris, 1981, 1991). A positive relationship may occur between biological control agents of weeds if attack by one agent enhances host nutritional quality (Masters et al., 2001; Utsumi & Ohgushi 2008; Johnson et al., 2009), causes changes in plant phenology that increase plant vulnerability to future attack (Miyamoto & Nakamura, 2004), or hinders host defenses thereby enabling attack by other agents (Losey & Denno, 1998; Prasad & Snyder, 2010; Stephens et al., 2013). A positive or neutral relationship between agents may increase the success of biological control efforts if multiple agents are released in tandem.

If a negative relationship (i.e., competition or amensalism) occurs between the agents, then releasing multiple agents could be less effective than releasing a single agent (Ehler & Hall, 1982; Leveque et al., 1993; De Moraes et al., 1999). This is especially true if the agent that is best suited to control the target species is the inferior competitor (Ehler & Hall, 1982; Leveque et al., 1993; Woodburn, 1996). Competition between herbivorous agents may occur if attack by one agent dramatically diminishes resources (Milbrath & Nechols, 2004), affects changes in plant phenology that decrease plant vulnerability to other agents (Milbrath & Nechols, 2004), or triggers host chemical defenses that prevent attack by other agents (Bezemer et al., 2003). If two competing species have high degrees of ecological similarity then interspecific competition in its most extreme form could result in competitive exclusion, wherein the inferior competitor is eliminated from the system (Hardin, 1960; Ehler & Hall, 1982; Denoth et al., 2002). However, if multiple species that occupy the same niche are able to separate their resource acquisition spatially or temporally (i.e., resource partitioning), then coexistence is possible (Schoener, 1982; Connell, 1980; James et al., 1992; Denno et al., 1995). Several meta-analyses have found that negative relationships among biological control agents and phytophagous insects are more common than positive relationships (Denno et al., 1995; Kaplan & Denno, 2007; Stephens et al., 2013). Additionally, a single biological control agent is often responsible for the suppression of a target pest (Myers et al., 1989; Denoth et al., 2002). Therefore, if the relationship between agents is negative, it may be more beneficial to release only the agent that is most successful at controlling the target species.

One such system with multiple herbivorous biological control agents consists of the nonnative species Russian knapweed (*Rhaponticum* (=*Acroptilon) repens*) and two gall-forming host-specific insects: the gall midge, *Jaapiella ivannikovi* (Diptera: Cecidomyiidae), and the gall wasp, *Aulacidea acroptilonica* (Hymenoptera: Cynipidae) (USDA APHIS 2008, 2009). Russian knapweed is a nonnative species that is widespread throughout the western United States (Jacobs & Denny, 2006). It primarily reproduces clonally through an extensive rhizomatic network

(Morrison et al., 1995) but can also travel to distant areas through seed dispersal (Djamankulova et al., 2008; Gaskin & Littlefield, 2017). To prevent its spread, the gall midge and gall wasp from Russian knapweed's native Eurasian range were approved for use as biological control agents in the United States. Galls are chambers formed of bloated plant tissues induced by certain insects, which offers protection and nourishment to the developing larvae. Interactions between the midge and the wasp are likely to be indirect and plant-mediated since these gall-forming insects can simultaneously gall a single ramet and spend the majority of their life cycle within their stationary galls. Once formed, galls act as metabolic sinks that can draw photosynthates towards the larvae and compete with other sinks, including meristematic root and shoot tips, fruit, and other galls (Inbar et al., 1995; Larson & Whitman, 1997; Dorchin et al., 2006; Kaplan et al., 2011; Hall et al., 2012).

Previous studies of Russian knapweed and its biological control agents have suggested that the prior establishment of midges may actually increase the establishment of wasps and the size of wasp galls (though midge gall size remained unchanged), potentially indicating a positive relationship between these two insects (Barosh et al., unpublished). This positive relationship may be due to the midge gall drawing photosynthates towards its location at the apical meristem, allowing wasp galls to intercept this increase in the flow of photosynthates because of their location on the stem (Barosh et al., unpublished) (Figure 1B). Previous research suggests that both biological control agents have a negative impact on Russian knapweed growth and reproductive capacity, particularly in its native Eurasian range (Djamankulova et al., 2008). These prior studies that examine the effects of both insect species together has occurred either in greenhouse conditions (Barosh et al., unpublished) or in the native range (Djamankulova et al., 2008).

In this study, the objectives were to determine the nature of the relationship between the midge and the wasp (e.g., positive, negative, or neutral) and the effects that each insect has on Russian knapweed in field conditions in its introduced range. Based on previous studies (Barosh et al., unpublished) and Colorado Department of Agriculture (CDA) observations (personal communication), I predicted that previous midge establishment would increase wasp establishment, but that prior wasp establishment would not impact midge establishment, resulting in a relationship of commensalism (+/0). I also predicted that both insects would have negative effects on Russian knapweed fitness in terms of growth and reproductive capacity. Since midges gall the apical meristem of the shoot and are multivoltine (i.e., producing multiple generations per year), I expected that they would have a greater negative impact on bud formation than wasps. I examined these questions experimentally by releasing the two insect species together and separately at 16 field sites throughout Colorado in the spring of 2019. To infer the nature of the relationship between the midge and wasp, I looked at the number of galls formed by each insect in the presence and absence of the other insect. I also measured aspects of Russian knapweed growth (height of ramets and the number of ramets) and reproductive capacity (the number of buds formed) to determine how each agent might impact plant fitness.

METHODS

Field Releases

Gall midges and gall wasps were released at sites with abundant Russian knapweed growth throughout Colorado, using a 2 x 2 factorial design with wasp and midge presence and absence. The releases occurred from the last week of April through the first week of May 2019 since the rapid growth of Russian knapweed and the warm temperatures this time of year increases the likelihood that the insects will survive and successfully gall the plant. There were a total of 16 sites consisting of four treatments each with four replicates: (1) midges released; (2) wasps released; (3) wasps and midges released; (4) a control in which no insects were released (Figure 2). Three of the four sites where both midges and wasps were released had midge establishment in prior years (AWRC, ARSN, DNS; Table 1). The average distance between neighboring sites with insect releases was about 3km in the Front Range region and 4km in the San Luis Valley region. The shortest distance between sites with releases was in the Front Range region at approximately 1km, but this was only for one pair of sites (DNN, DNS; Table 1). Latitude and longitude for each site were obtained using a Garmin etrex 20x GPS navigator (Garmin International Inc., Olathe, KS) (Table 1).

Both insects were obtained from the CDA Insectary in Palisade, CO. They were shipped overnight and kept refrigerated at 1.6°C until released within 48 hours. Wasps were released as adults and midges were released as larvae in their galls. Each wasp release consisted of ~200 adult wasps, and each midge release consisted of ~300 midge individuals (25 midge galls with an average of 12 midges emerging from each gall) (Meyers et al., 2015). This amounts to approximately 150 female midges and 180 female wasps per release (Djamankulova et al., 2008). Previous studies have shown that these numbers are sufficient for successful establishment (Barosh, 2020).

Two 16m transects that intersected perpendicularly at their midpoints were placed within each of the 16 sites (Figure 3A). One sampling frame (0.5m x 0.5m) was placed at the center of the intersection and five sampling frames (also 0.5m x 0.5m) were placed along each of the 4 arms of the transects at the 1m, 2m, 4m, 6m, and 8m marks as measured from the center for a total of 21 sampling frames per site (Figure 3A). Insects were released in the center frame at the intersection of the two transects. Sampling frames were more closely spaced near the center of the bisecting transects since I expected that more insects would establish closer to the release point.

All 16 sites were measured twice during the summer of 2019, once at the beginning of May and once at the end of July. At each site, the data collected per sampling frame included: the height (measured from the base of the stem at soil level to the apical meristem) of the tallest ramet, the height of a randomly selected ungalled ramet, the total number of ramets, the total number of flower buds, the number of ramets galled by each insect, the number of midge galls per galled ramet, and the number of wasp galls per galled ramet.

Data Analysis

Analyses were conducted using R version 4.0.4 (R Core Team, 2021) and the R packages lme4 (Bates et al., 2015), emmeans (Lenth, 2020) and MuMIn (Bartoń, 2020). Plots were generated using ggeffects (Lüdecke, 2018), and ggplot2 (Wickham, 2016).

Normality of the data was confirmed using Quantile-Quantile plots and histograms and equal variance was confirmed using residuals vs. fitted plots. The data in all selected models was either within a Cook's distance of 1, indicating that there were no points of high influence, or the results were confirmed in a separate analysis with the points of high influence removed. The results were confirmed in all cases except for one that had wasp galls as a response variable and midge galls as a predictor variable, which is discussed further below.

Since the wasp and midge galls were count data, a generalized linear mixed model (GLMM) with a Poisson distribution was used to determine the impact that midge establishment might have on wasp establishment. The model included the number of wasp galls as a response variable and the number of midge galls as a predictor variable. The number of ramets per plot, the height of a random ungalled ramet per plot, and site were added as co-variates. Site was treated as a random variable while all other terms were treated as fixed. The data included plots from all sites where wasps were released, for a total of 168 sampling frames (8 sites x 21 frames per site). Seven points of high influence were found and the same model was run with each of these points individually removed, with one point from a center frame with high densities of insects altering the results.

To determine the impact that wasp establishment might have on midge establishment, a GLMM with a Poisson distribution was also used with midge galls as the response, wasp galls as the predictor and the same co-variates as in the previous model. This analysis was conducted on a sampling frame level, which reflects the level at which the data were collected. The data included sampling frames from all seven sites where midges were released and where Russian knapweed persisted throughout the season (N=146).

To determine the nature of the intraspecific relationships, log likelihood ratio tests were used to see if there was a difference in the expected versus observed values for each insect species forming only one gall per galled plant or multiple galls per galled plant.

To determine the impact of the galling insects on plant fitness, linear mixed models (LMMs) were constructed that examined the effects of midge galls and wasp galls on the number of buds formed per sampling frame, the height of ramets within sampling frames, and the change in the number of ramets per sampling frame. When the number of buds formed per sampling frame was considered as the response variable, a log transform on the response and predictor variables was used to satisfy model assumptions. The predictor variables were the number of midge galls per sampling frame, the number of wasp galls per sampling frame, and the interaction of wasp and midge galls per sampling frame. The co-variates were the number of ramets per sampling frame, the height of a random ungalled ramet per sampling frame and site. Site was considered as a random variable, while all other terms were considered as fixed. In accordance with how the data was collected, this analysis on the number of buds was also performed on a sampling frame level. All sampling frames (except a few that had no ramets) in all sites where Russian knapweed persisted throughout the season were included in this analysis (N = 300).

The height of galled and ungalled ramets within sampling frames was considered as a response variable to determine how wasp galls and/or midge galls were associated with height. When the height of ramets was treated as the response variable, a LMM was used that included the number of midge galls per ramet, the number of wasp galls per ramet and the interaction of midge and wasp galls per ramet as fixed predictor variables as well as plots nested within sites as a random co-variate. This analysis was performed on a ramet level, which reflects that the data on height was collected on multiple ramets per sampling frame. The data for this analysis consisted of one ungalled ramet per sampling frame and all the galled ramets found per sampling frame across all 15 sites where Russian knapweed persisted (N=502).

The change in the total number of ramets per plot over the season was considered as a response variable to determine how the formation of galls of either species was associated with the amount of ramet growth over the season. The change in the number of ramets per sampling frame was calculated by taking the difference in the number of ramets between the first and last visit. A LMM was constructed that used the fixed predictor variables of the number of midge galls per sampling frame, the number of wasp galls per sampling frame, the interaction between the number of midge and wasp galls per sampling frame and the height of a random ungalled ramet per sampling frame, with site was treated as a random variable. This analysis included sampling frames across all sites where Russian knapweed persisted for a total of 15 sites (N=315).

To get a sense of how successfully the insects were able to establish, establishment rates on a site level were calculated as the number of sites that had galls of a given insect present at the time of the last visit divided by the total number of sites where releases of that insect occurred.

Marginal R^2 (the variance explained only by fixed effects) and Conditional R^2 (the variance explained by the overall model, including random and fixed effects) values were calculated for each model using the MuMIn package (Bartoń, 2020), which follows methods developed by Nakagawa et al. (2017). It is worth noting that these values are typically much lower than R^2 values for non-mixed linear models due to variation between levels of random effects (Nakagawa & Schielzeth, 2013).

RESULTS

Overview of insect establishment

By late July of 2019, insects established at 10 of the 12 sites that were treated with either midges only, wasps only, or midges and wasps (Figure 4). Establishment is defined by the presence of galls of a given species. Midges established at a total of 8 sites: 3 from the midge and wasp treatment, 3 from the midge only treatment, and 2 from the wasp only treatment where midges migrated to by late July. Wasps established at a total of 6 sites: 3 from the midge and wasp treatment and 3 from the wasp only treatment. There was strong establishment (>50 galls of a given species) at 3 sites, which included one from each of the 3 treatments where insects were released. There was moderate establishment (25-50 galls) at one site, where both midges and wasps were released. There were low levels of establishment (<25 galls) at 6 sites, which consisted of 2 sites from each of the 3 treatments where insects were released. Two sites experienced no establishment by this time. In one of the sites with no establishment, which was in the midge only treatment, the knapweed dried up and died completely by July 2019 and was not found at all in July 2020. Since no chemical herbicides were used and this site was noticeably dry, we interpret this die-off as being caused by drought.

Averaging across all sites that had releases of a given insect, the average percent of ramets galled by wasps per site was 1% (with a range of 0 % to 7%) and the average percent of ramets galled by midges per site was 6% (with a range of 0.2% to 63%). There were up to six wasp galls found on a single ramet, with two being the modal number of wasp galls per ramet. There were up to seven midge galls found on a single ramet, with one being the modal number of midge galls per ramet. There was only one ramet across all sites that had both a midge gall and a wasp gall.

The interspecific relationship

An increased presence of midges was associated with a decreased presence of wasps, indicating a negative interspecific relationship (Figure 5). For every additional 10 midge galls, the number of wasp galls is expected to decrease by a factor of 0.45 (ß = -0.08, p = 0.007, 95% CI: -0.14, -0.02) (Figure 5). Additionally, across all sites with both midge and wasp releases there was only one observation of a midge gall and wasp gall on the same ramet and only four sampling frames that had both midge galls and wasp galls. Seven points of high influence were found in this analysis. Six of the seven points of high influence were retained since they did not impact the results when individually removed from the analysis. However, one point with an unusually high number of wasp galls in the sampling frame (the center frame of a midge and wasp site in the Front Range) caused a change in the results. Once this point was removed, the relationship between midge galls and wasp galls was found to no longer be significant though the negative direction was the same (ß = -0.07, p = 0.22, 95% CI: -0.17, 0.04) (Figure 6). Since the multivoltine midges emerge and form galls throughout the summer, there is the potential for them to respond to the wasps that formed galls soon after release at the end of spring. However, when midge galls were considered as a response variable, the effect of wasp galls was not significant (ß = -0.03, p = 0.13, 95% CI: -0.07, 0.001) (Figure 7).

The intraspecific relationships

There is evidence for a negative intraspecific relationship among midges as they were more likely to form single galls per midge-galled ramet than multiple galls (92/150 vs. 58/150; likelihood ratio χ^2 = 3.91, d.f. = 1, p = 0.048). Wasps may have a neutral intraspecific relationship as they were just as likely to form single galls per wasp-galled ramet as multiple galls (27/61 vs. 34/61; likelihood ratio χ^2 = 0.403, d.f. = 1, p = 0.53).

Russian knapweed growth and reproductive capacity

Neither wasps (ß = -0.06, p = 0.70, 95% CI: -0.36, 0.24) (Figure 8A) nor midges (ß = -0.18, p = 0.22, 95% CI: -0.47, 0.11) (Figure 8B) reduced the number of flower buds. A summary of the number of buds formed by site can be found in figure 9.

Greater numbers of midge galls were associated with taller ramets (ß = 0.94, p = 0.01, 95% CI: 0.19, 1.68) (Figure 10A). Specifically, the formation of each additional midge gall corresponded with an increase of 0.94cm in ramet height. No significant relationship was found between the height of ramets and the number of wasp galls (ß = 0.47, p = 0.38, 95% CI: -0.62, 1.56) (Figure 10B). A summary of the height of random ungalled ramets per site can be found in figure 11.

Greater amounts of midge galls were associated with a greater change in the number of ramets over the course of the season (ß = 0.39, p = 0.03, 95% CI: 0.04, 0.75) (Figure 12A). Specifically, each additional midge gall was associated with an increase of 0.39 ramets per sampling frame. The change in the number of ramets per sampling frame was not significantly associated with the number of wasp galls (ß = -0.06, p = 0.78, 95% CI: -0.47, 0.35) (Figure 12B).

DISCUSSION

The aims of this study were to determine the nature (e.g. positive, negative, or neutral) of the indirect plant-mediated interactions between two phytophagous insects that are used as biological control agents and the effects of these agents on the growth and reproductive capacity of the target plant. Contrary to my prediction that midge establishment would increase wasp establishment, it appears that midge establishment may have a negative (Figure 5) or neutral (Figure 6) impact on wasp establishment. I further predicted that both agents would reduce the growth and reproductive capacity of Russian knapweed, which would be indicated by reductions in flower bud (and ultimately seed) production, height, and new ramet formation. However, neither agent reduced the number of flower buds (Figure 8), and midge establishment was associated with both taller ramets (Figure 10) and the growth of more ramets per sampling frame over the course of the season (Figure 12).

The interspecific relationship

The potentially negative or neutral association between the establishment of wasp galls based on the establishment of midge galls on a sampling frame level does not support the hypothesis of commensalism existing between the two species, wherein the establishment of midges would promote the establishment of wasps. From this data, it appears that if a relationship does exist between the two insect species it may be a negative relationship, such as competition (-/-) or amensalism (-/0). A study that examined several cases of multiple natural enemies attacking host plants found that antagonistic effects were more common when plant reproductive structures were attacked, specifically by insects in the order Diptera and with plants in the family Asteraceae (Stephens et al., 2013). According to optimal defense theory, plants concentrate defense compounds among valuable parts like reproductive structures (Zangerl & Rutledge, 1996). Therefore, damage to reproductive structures could generate defense compounds that would make it more difficult for subsequent enemies to attack the same plant, resulting in negative interspecific interactions (Stephens et al. 2013).

The possibility of a negative relationship is also supported by the fact that throughout the summer of 2019, there was only one observation of midges and wasps establishing on the same ramet and only four observations of midges and wasps establishing within the same sampling frame. That the insect species seem to establish away from each other may indicate that resource partitioning is occurring, which could allow the two competitors to co-exist (Connell, 1980; Schoener, 1982; Denno et al., 1995). The presence of wasp galls does not appear to influence the presence of midge galls, which agrees with this portion of our initial hypothesis. These results may be evidence for either an amensalist (-/0) or neutral (0/0) relationship existing between the two species at a sampling frame level.

This outcome contrasts with previous studies that suggested that the presence of midges may actually increase the likelihood of gall formation by wasps, indicating a positive relationship in at least one direction. A previous greenhouse choice study that allowed wasps to choose between midge-galled and ungalled ramets, found that wasps were 2.3 times more likely to oviposit on the midge-galled ramets (Barosh et al., unpublished). In addition, wasp galls grew larger on ramets that had been previously galled by midges, while midge gall size remained unaffected (Barosh et al., unpublished). This study by Barosh et al. (unpublished) was conducted on the level of individual ramets such that insects were only able to gall ramets in separate pots that lacked rhizomatic connections. It is possible that a positive relationship may exist between the insects on the scale of an individual ramet since the midge gall could draw resources upward towards the apical meristem, allowing for interception by the wasp gall (Figure 1B). However, when insects gall separate ramets within a plot they may compete for plant resources by pulling photosynthates through the rhizomatic network towards the ramets that they occupy (Figure 1A).

The point of high influence in the present study that altered the results from showing a negative and significant association between wasps and midges to showing no significant association between the two species is from a sampling frame with 51 wasps and only 2 midges despite it being a center frame where both insect species were released. It may be that multi-sink competition only becomes noticeable when there is a higher density of sinks that occur on separate but nearby ramets (Pineda et al., 2017). The magnitude and the direction of the relationship between midges and wasps may also be dependent upon the availability of water and nutrients. Perhaps with greater water and nutrient availability in greenhouse experimental conditions, the relationship may become more positive, whereas the limited water and nutrient availability in the field may lead to greater interspecific competition.

The intraspecific relationships

The fact that midges were more likely to form single galls per galled ramet than multiple galls (92/150 vs. 58/150; likelihood ratio $\chi^2 = 3.91$, d.f. = 1, p = 0.048) suggests that intraspecific competition may be occurring. This corresponds with other research that also found negative intraspecific interactions among midges, with midge galls being smaller and containing fewer larvae when there were more midge galls per plant (Barosh, 2020). Therefore, midges may prefer to disperse in the field and avoid forming multiple galls per plant. This competition may be heightened by the limited amount of water and nutrients in field conditions, allowing competition to occur even at low densities. Wasps were just as likely to form single galls per galled ramet as multiple galls (27/61 vs. 34/61; likelihood ratio $\chi^2 = 0.403$, d.f. = 1, p = 0.53), which indicates a neutral intraspecific interaction may be occurring.

Russian knapweed growth and reproductive capacity

The positive relationship between height and midge galls may simply be due to the midges choosing ramets that are more “fit” and thus able to grow taller and/or more rapidly. Other field studies in both Uzbekistan (Djamankulova et al., 2008) and Colorado (Barosh, 2020) have found that the formation of midge galls is associated with a significant decrease in height. However, a greenhouse study that used Russian knapweed from Colorado showed an increase in aboveground biomass associated with the formation midge galls, with plants that were exposed to 3 midges exhibiting 15% greater biomass, and plants that were exposed to 6 midges exhibiting 60% greater biomass (Barosh, 2020).

The positive relationship between the change in the number of ramets per sampling frame and the presence of midge galls may simply be due to the midges having access to increased galling locations. This trend may be visible for midge establishment and not wasp establishment because midges are multivoltine, allowing them to gall new ramets that grow throughout the season. However, there is the possibility that attack by midges may stimulate Russian knapweed to allocate resources to belowground growth and perhaps the production of new ramets. The Barosh (2020) greenhouse study indicates that attack by midges is associated with an increase in the number of root buds per plant and the belowground biomass. This study also found that plants exposed to midges had a greater number of ramets, though this result was not significant (Barosh, 2020). Similarly, a field experiment in Colorado found increased Russian knapweed ramet growth after attack by midges (Barosh, 2020).

There were no significant effects found for either insect on bud formation of Russian knapweed on a sampling frame level. This contradicts the prediction that the establishment of these insects would decrease bud formation, which has been found in previous studies (Djamankulova et al., 2008). The establishment of midges in particular was expected to dramatically decrease bud formation both because they gall the apical meristem and produce multiple generations of galling females per season. Although seed production was not measured in this study, it is likely that since there was no significant effect on bud formation, then there was not a significant effect on seed production. Bud formation was positively associated with the height of ungalled Russian knapweed and the total number of ramets present in a plot. These are likely indicators of the health or fitness of the knapweed within a given plot, with healthier knapweed forming more buds, growing taller, and producing more ramets.

It is important to note that the densities of the insects at these sites in Colorado and within the United States generally is unsurprisingly much lower than in their native Eurasian range. In the native range of Uzbekistan there can be up to 12 wasp galls on one ramet, with densities as high as 400 wasp galls per 100 ramets (Schaffner, unpublished results, as cited in Djamankulova et al., 2008). In the same region, a maximum of 15 midge galls have been observed on one ramet, but less than 10% of ramets were found to have midge galls (Schaffner, unpublished results, as cited in Djamankulova et al., 2008). In contrast, low densities of midges have been observed in the non-native range of Wyoming, where the percent of ramets galled by midges at 100 permanent plots was determined to be only 1% to 2%, even four years after release, and no significant impacts on Russian knapweed were observed (Meyers et al., 2015). In our study, the average percent of ramets galled by wasps (1%) and midges (6%) as well as the number of wasp galls per ramet (max = 6, mode = 2) and the number midge galls per ramet (max = 7, mode = 1) was also low compared to the native range. It is possible that the lack of significant negative effects on Russian knapweed bud formation, height and new ramet production is due to the relatively low densities of these insects in their non-native range.

Conclusions and future directions

When comparing the results of this study and previous studies, there appears to be differing evidence on the type of interaction that occurs between the midge and the wasp as well as how each insect impacts Russian knapweed growth and reproductive capacity. However, these differences may be due to the interactions between the insects occurring at different scales, with positive relationships occurring when insects establish on the same ramet and competitive relationships occurring when insects establish between ramets. These differences may also be due to a variety of factors, such as the density of the insects in different studies, potential variation in Russian knapweed in its native versus its introduced range, and the multitude of variables that arise in field conditions as opposed to greenhouse studies. In particular, the availability of water could be a factor that determines whether or not the interaction between the midge and the wasp is positive or negative, with greater water availability in the greenhouse enabling a positive relationship and limited water availability in the field promoting a negative relationship. A similar pattern may occur with availability of soil nutrients, which are abundant in potting soil but limited in natural conditions. Future studies that manipulate specific amounts of water and nutrient availability should be conducted to gain a better understanding of the impacts on Russian knapweed and the interactions between the insects. Considering the univoltine nature of the wasp and the lower densities of both insects over one field season, it would be useful to conduct studies that last for multiple seasons both in the field and in the greenhouse.

Determining the nature of the interaction between multiple agents is critical to the success of biological control programs and as such should be considered in testing protocols. The need for more studies of this kind is particularly important in light of relatively new information on the prevalence of indirect plant-mediated interactions that have traditionally been overlooked both in biological control programs and in ecological studies more generally. There is the potential for the study of countless interactions between herbivores that may be vital to understanding how communities function.

TABLES AND FIGURES

Table 1. List of site codes, locations, and releases performed at each site.

Code	Name	Region	County	Latitude	Longitude	Releases
ARSN	Ardec Site North	Front Range	Larimer	N 40°40.473'	W 104°58.928'	Midge & Wasp
ARSS	Ardec Site South	Front Range	Larimer	N 40°40.430'	W 104°58.922'	Control
AWNC	Alamosa Wildlife Refuge North Central	San Luis Valley	Alamosa	N 37°27.495'	W 105°46.585'	Wasp
AWRC	Alamosa Wildlife Refuge Central	San Luis Valley	Alamosa	N 37°27.380'	W 105°49.591'	Midge & Wasp
AWRN	Alamosa Wildlife Refuge North	San Luis Valley	Alamosa	N 37°27.191'	W 105°44.806'	Midge
AWRW	Alamosa Wildlife Refuge West	San Luis Valley	Alamosa	N 37°27.447'	W 105°49.978'	Control
BLN	Barbara's Land North	San Luis Valley	Alamosa	N 37°33.034'	W 105°51.012'	Control
BLS	Barbara's Land South	San Luis Valley	Alamosa	N 37°32.851'	W 105°51.157'	Midge & Wasp
DNN	Denver Naturalists North	Front Range	Adams	N 39° 50.407'	W 104°46.567'	Wasp
DNS	Denver Naturalists South	Front Range	Adams	N 39° 50.035'	W 104°46.598'	Midge & Wasp
ELT	Excel Lake Thomas	Front Range	Weld	N 40° 13.157'	W 104°56.804'	Wasp
ESV	Excel St. Vrain	Front Range	Weld	N 40° 14.350'	W 104°53.594'	Midge
SCN	Second Creek North	Front Range	Adams	N 39° 52.639'	W 104°47.594'	Control
SCS	Second Creek South	Front Range	Adams	N 39° 52.321'	W 104°47.513'	Midge
TLN	Todd's Land North	San Luis Valley	Alamosa	N 37°29.290'	W 105°46.165'	Wasp
TLS	Todd's Land South	San Luis Valley	Alamosa	N 37°28.483'	W 105°46.297'	Midge

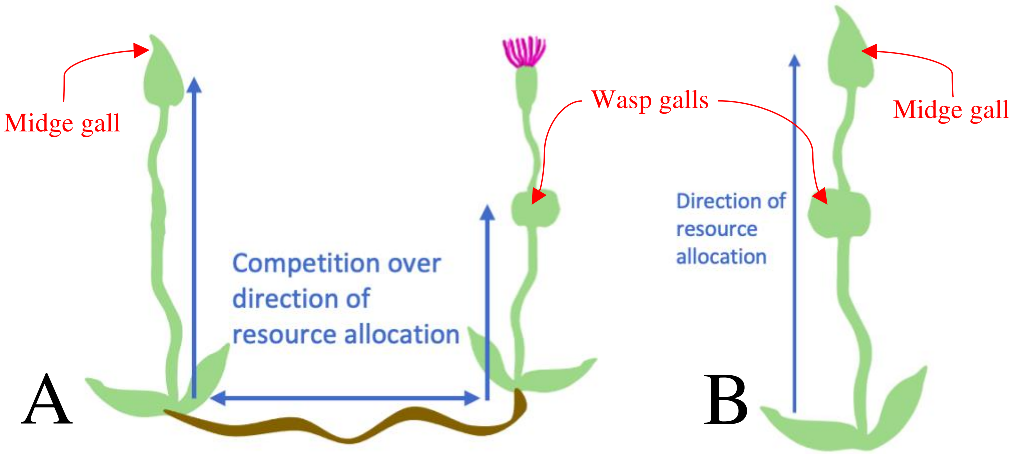

Figure 1. There may be a negative relationship between insects on separate ramets (A) and a positive relationship between insects on the same ramet (B) depending upon how the midge galls and wasp galls affect resource allocation as sinks.

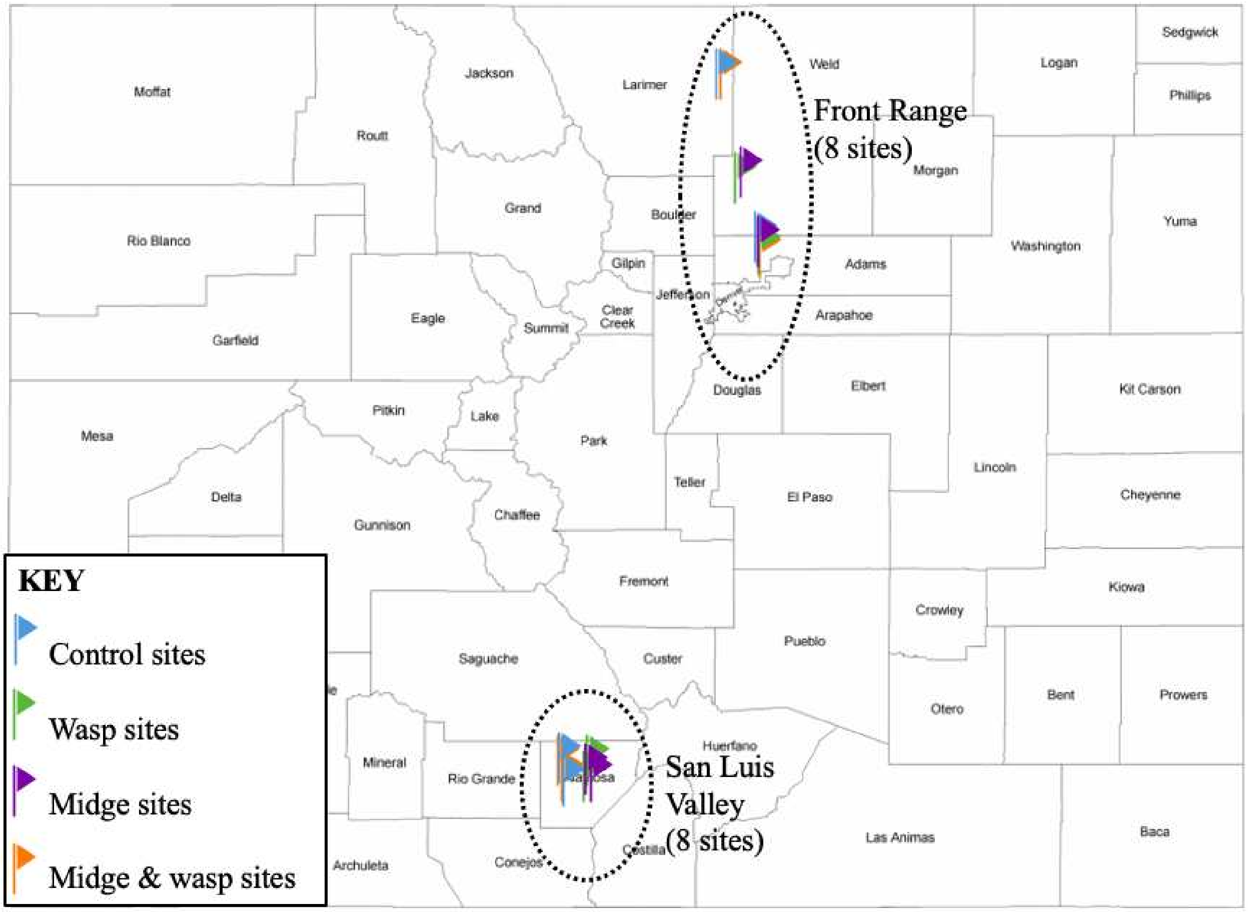

Figure 2. Map of site locations based on treatment. There were 8 sites (2 from each treatment group) in both the Front Range and the San Luis Valley regions of Colorado. All the Russian knapweed died off at a midge-only release site (SCS) in Adams County in the Front Range cluster of sites. See Table 1 for a description of all sites.

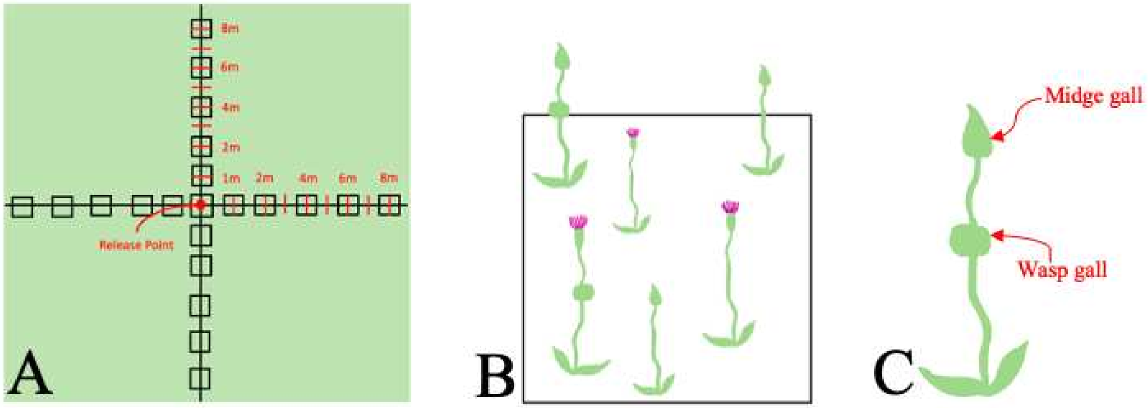

Figure 3. There are three different scales to consider. (A) The site level scale (each site consists of 21 sampling frames with insect releases performed at the center of the intersecting transects). (B) The sampling frame level scale (each sampling frame is 0.5m x 0.5m). (C) The individual ramet scale (there may be multiple individual ramets within a single sampling frame).

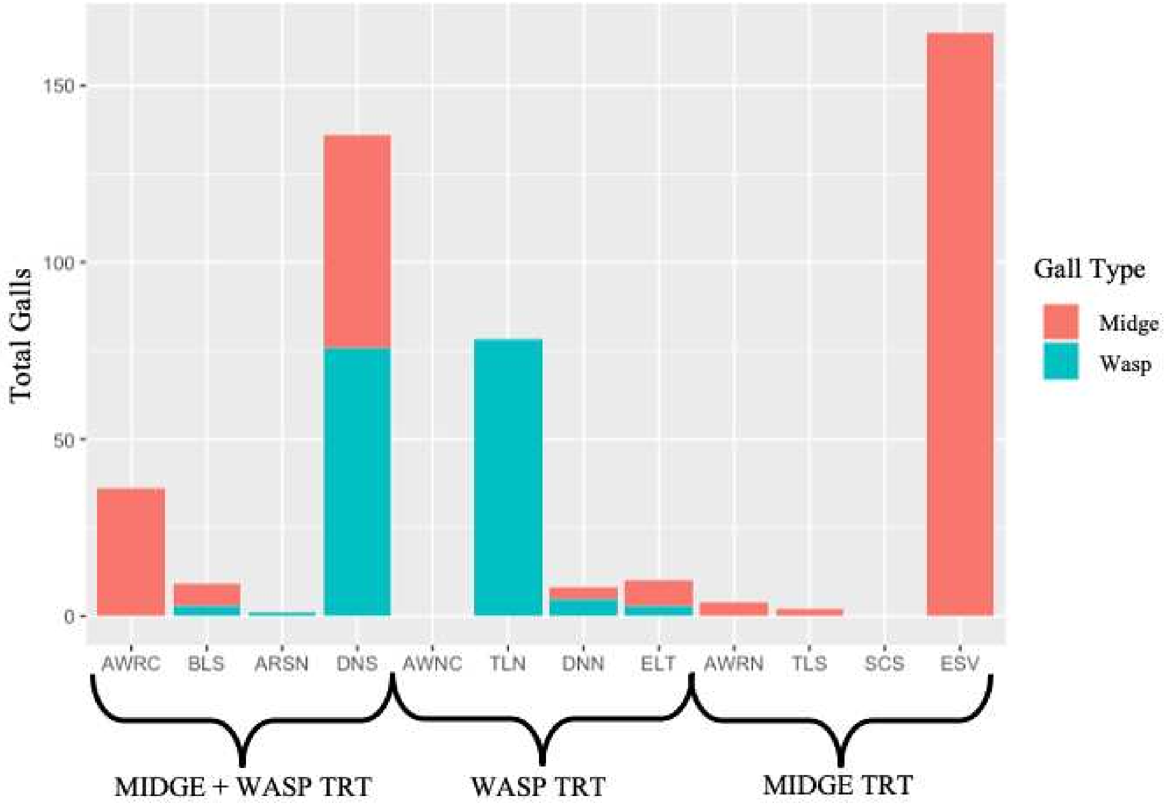

Figure 4. Total numbers of midge galls (red) and wasp galls (blue) by site in July. Sites are grouped by treatment. All the Russian knapweed died off at the site labeled “SCS,” which was in Adams County in the Front Range cluster of sites. See Table 1 for a description of all sites.

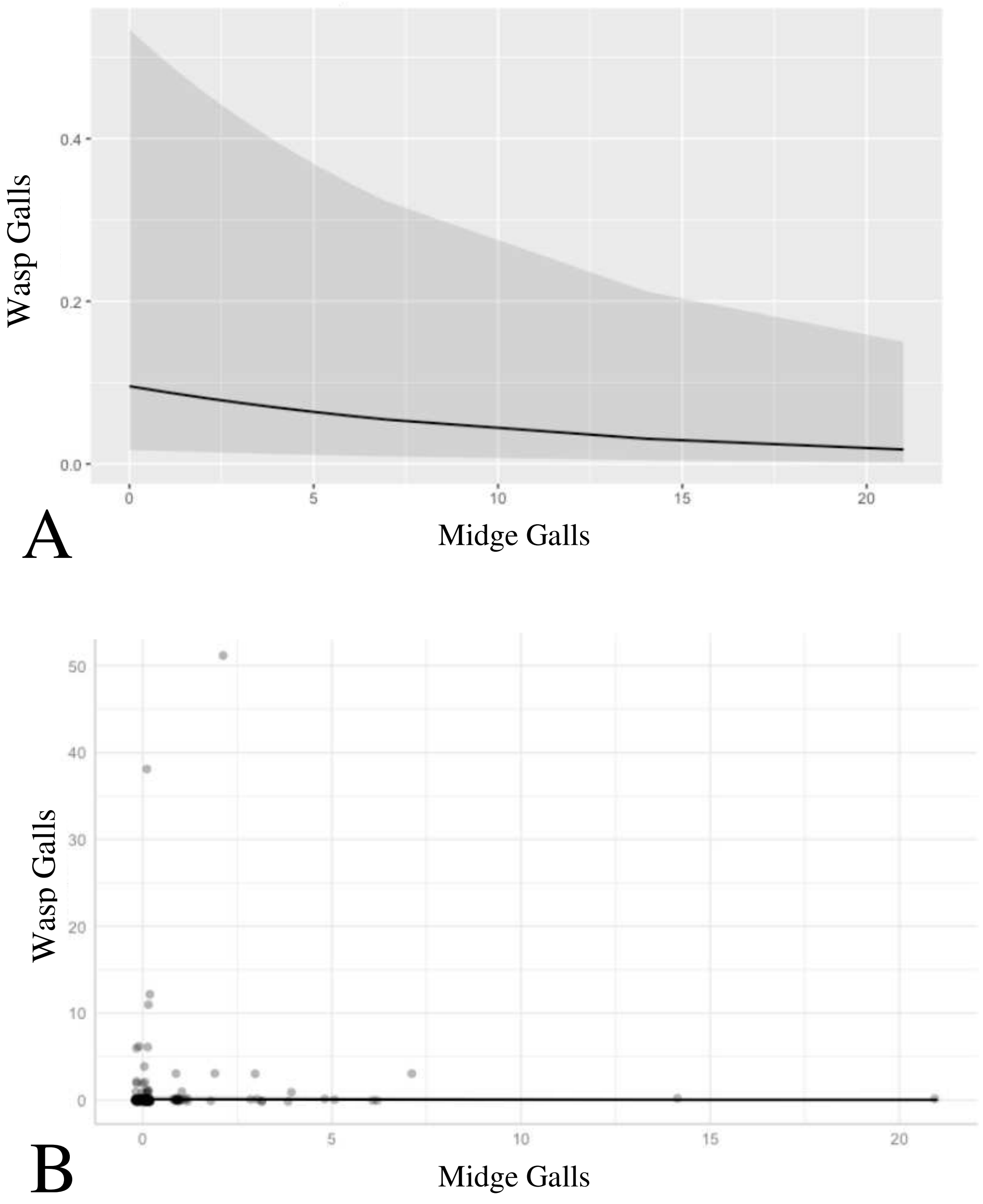

Figure 5. Plots of model-based predictions of the number of wasp galls with respect to the number of midge galls (line with standard error shaded in gray). The scale of observation is on the sampling frame level across all 8 sites with wasp releases (N = 168). ß = -0.08, p = 0.007, 95% CI: -0.14, -0.02, Marginal R^2 = 0.17, Conditional R^2 = 0.89. (A) Plot on the log count scale used in the Poisson model. (B) Plot on the scale of the data (points).

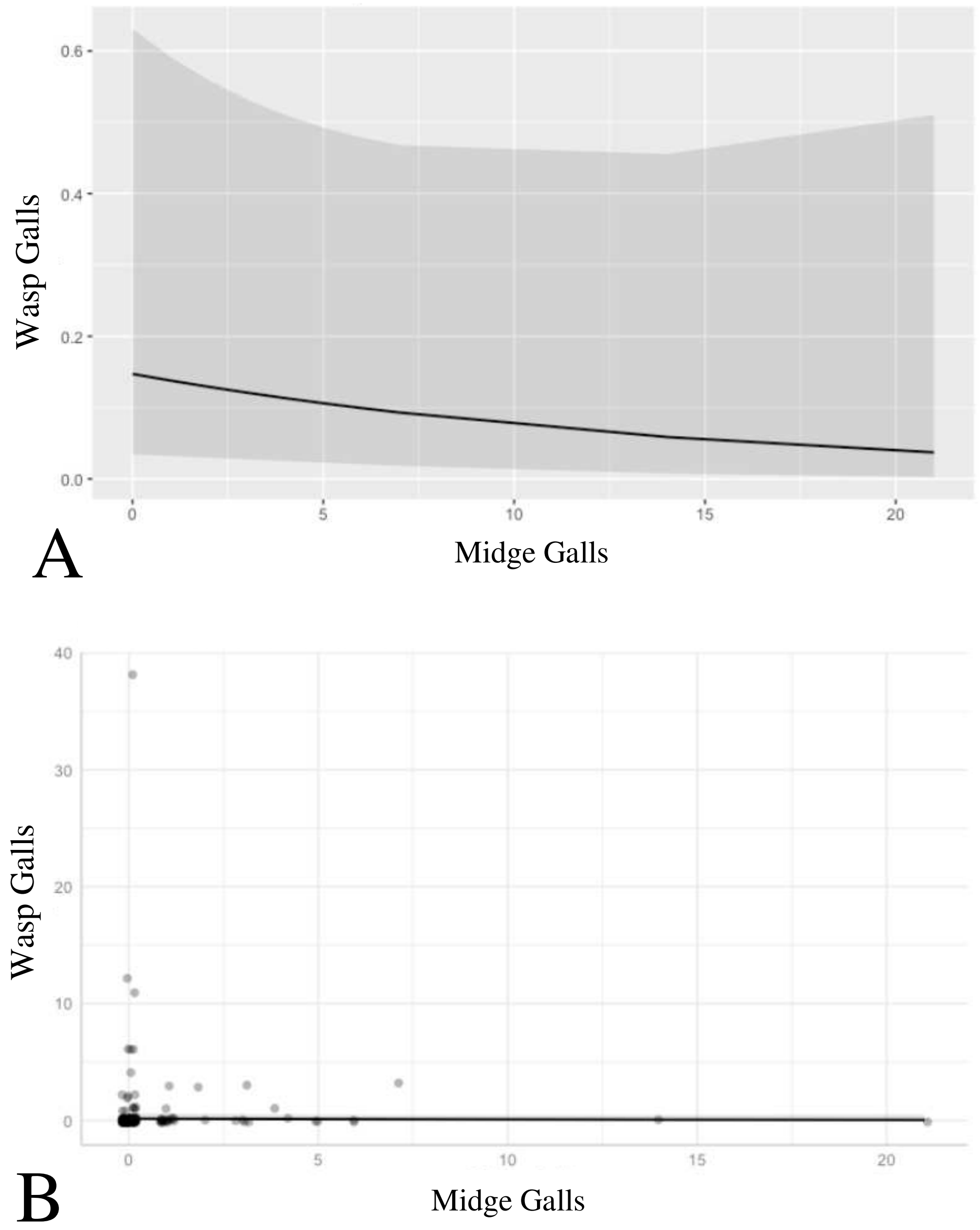

Figure 6. Plots of model-based predictions of the number of wasp galls with respect to the number of midge galls (line with standard error shaded in gray) with the critical point of high influence removed (all other data is identical to that presented in Figure 5). Removing this critical point of high influence yields a non-significant result; the plots shown here are for comparison with the significant result in Figure 5. The scale of observation is on the sampling frame level across all 8 sites with wasp releases (N = 167). ß = -0.07, p = 0.22, 95% CI: -0.17, 0.04, Marginal R^2 = 0.01, Conditional R^2 = 0.67. (A) Plot on the log count scale used in the Poisson model. (B) Plot on the scale of the data (points).

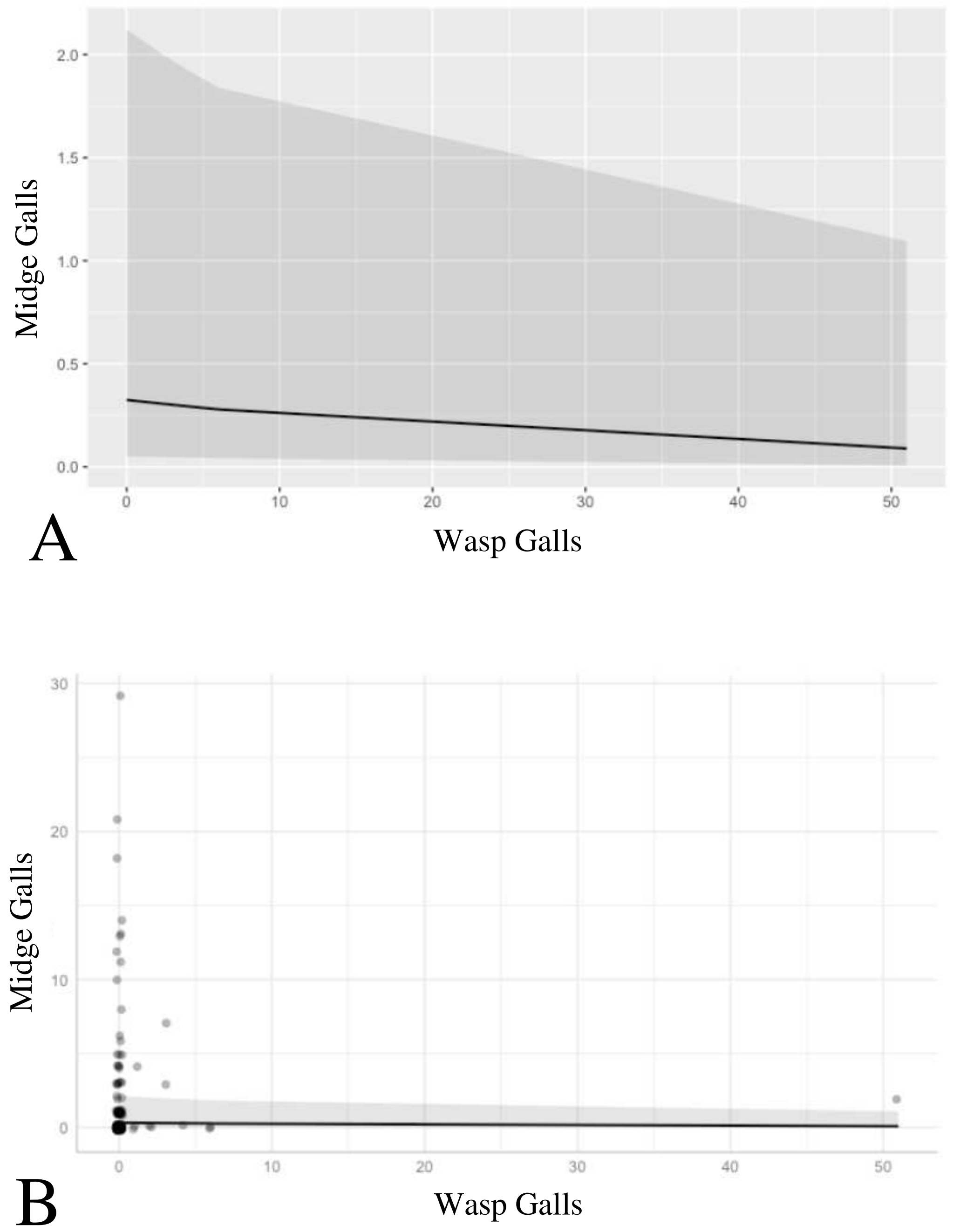

Figure 7. Plots of model-based predictions of the number of midge galls with respect to the number of wasp galls (line with standard error shaded in gray). The scale of observation is on the sampling frame level across all 8 sites with midge releases (N = 146). ß = -0.03, p = 0.13, 95% CI: -0.07, 0.001, Marginal R^2 = 0.13, Conditional R^2 = 0.95. (A) Plot on the log count scale used in the Poisson model. (B) Plot on the scale of the data (points).

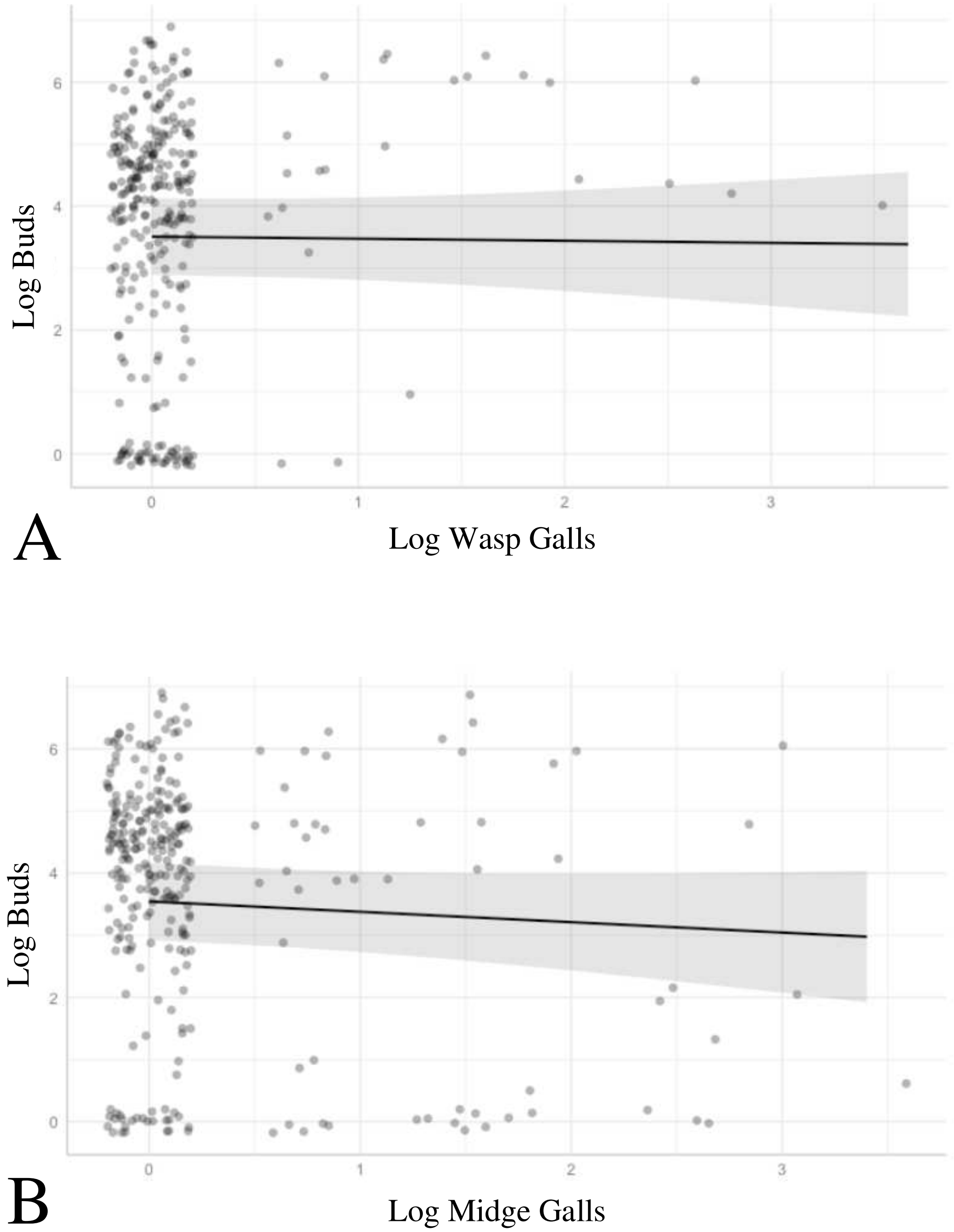

Figure 8. Plots of model-based predictions of the number of buds with respect to the number of galls of both species (line with standard error shaded in gray) with log transformed data (points). The scale is on the level of the sampling frame. Across all 15 sites, with 21 sampling frames per site, there were a total of 300 sampling frames that had at least one ramet growing (N=300). Marginal $R^2 = 0.21$, Conditional $R^2 = 0.70$. (A) Predictions of buds based on wasp galls (ß = -0.06, p = 0.70, 95% CI: -0.36, 0.24). (B) Predictions of buds based on midge galls (ß = -0.18, p = 0.22, 95% CI: -0.47, 0.11).

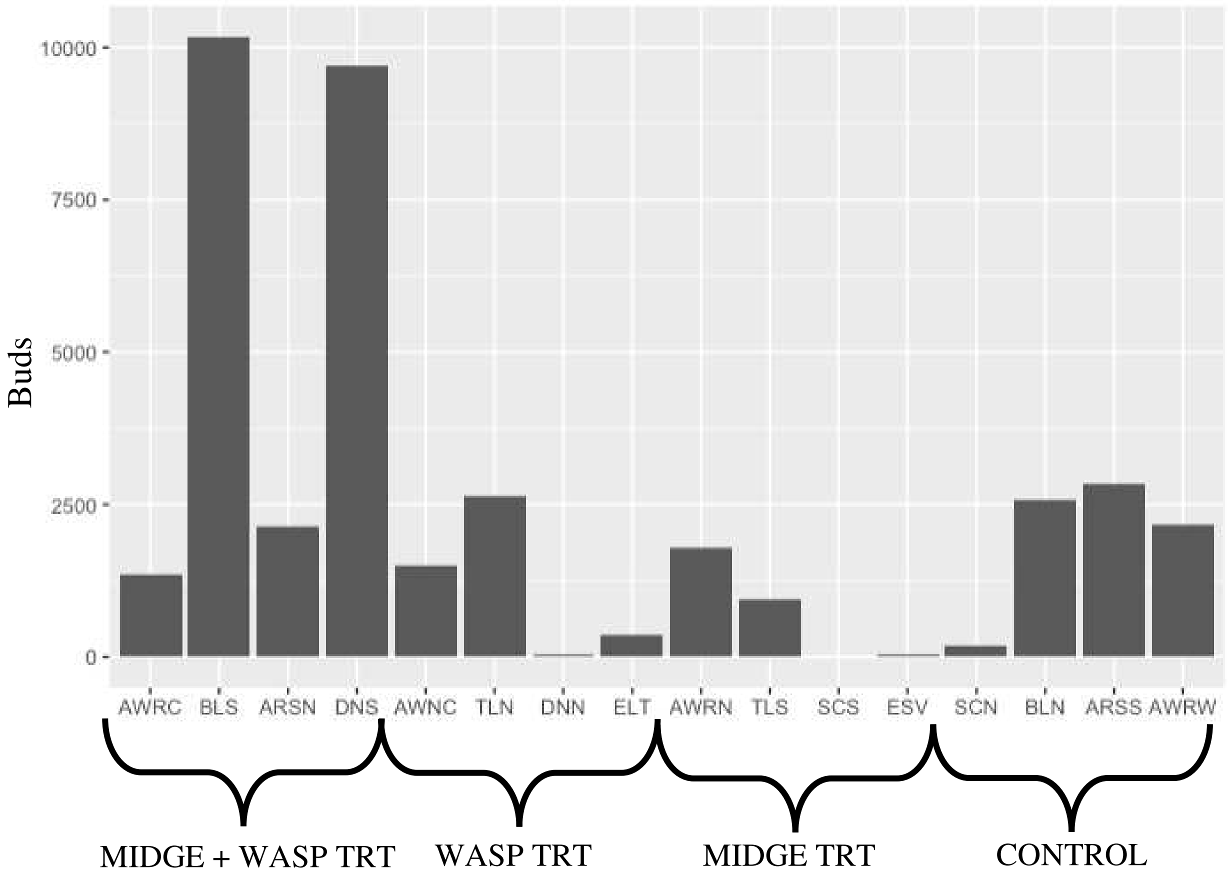

Figure 9. Total amounts of buds by site. Sites are grouped by treatment. All the Russian knapweed died off at the site labeled "SCS," which was in Adams County in the Front Range cluster of sites. See Table 1 for a description of all sites.

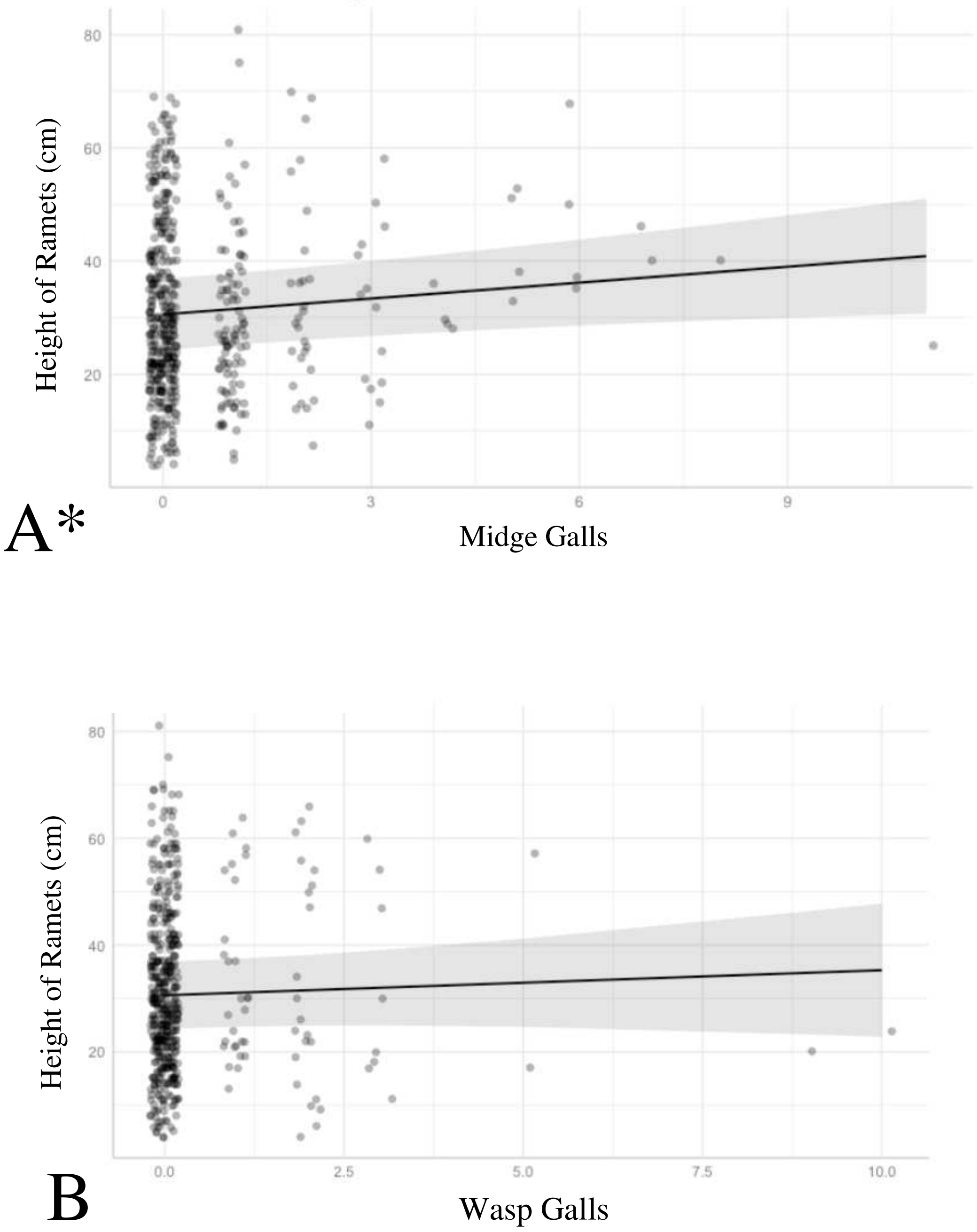

Figure 10. Plots of model-based predictions of the height of ramets with respect to the number of galls of both species (line with standard error shaded in gray) with data (points). The scale is on the level of individual ramets. The data includes one random ungalled ramet per sampling frame and however many ramets had galls of either insect per sampling frame. Across all 15 sites, with 21 sampling frames per site, there were a total of 300 plots that had at least one ramet growing and a variable number of additional galled ramets (N = 502). Marginal $R^2 = 0.01$, Conditional $R^2 = 0.74$. (A) Predictions of height of ramets based on midge galls. This relationship is significant (ß = 0.94, p = 0.01, 95% CI: 0.19, 1.68). (B) Predictions of height of ramets based on wasp galls (ß = 0.47, p = 0.38, 95% CI: -0.62, 1.56).

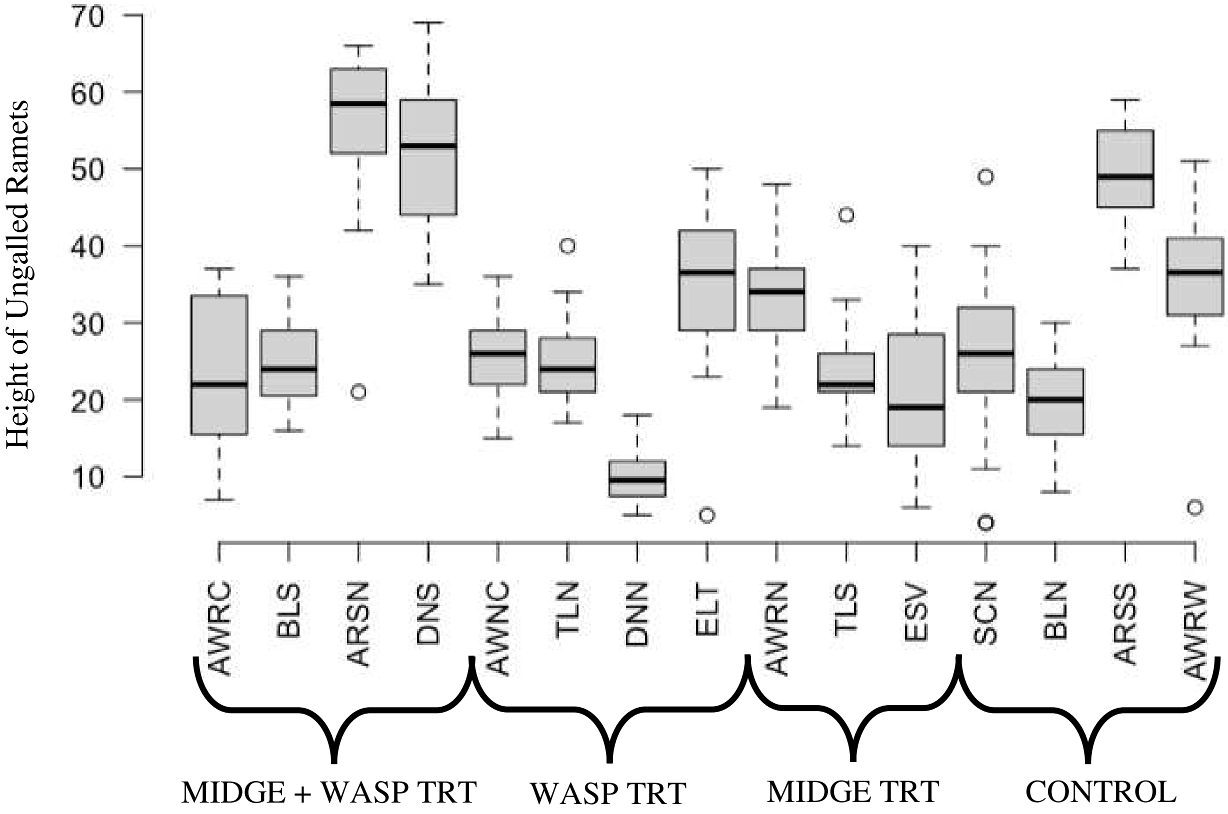

Figure 11. Height of randomly selected ungalled ramets across sites that are grouped by treatment. Since there were no ramets from the midge only treated site "SCS," it was excluded from the plot. See Table 1 for a description of all sites.

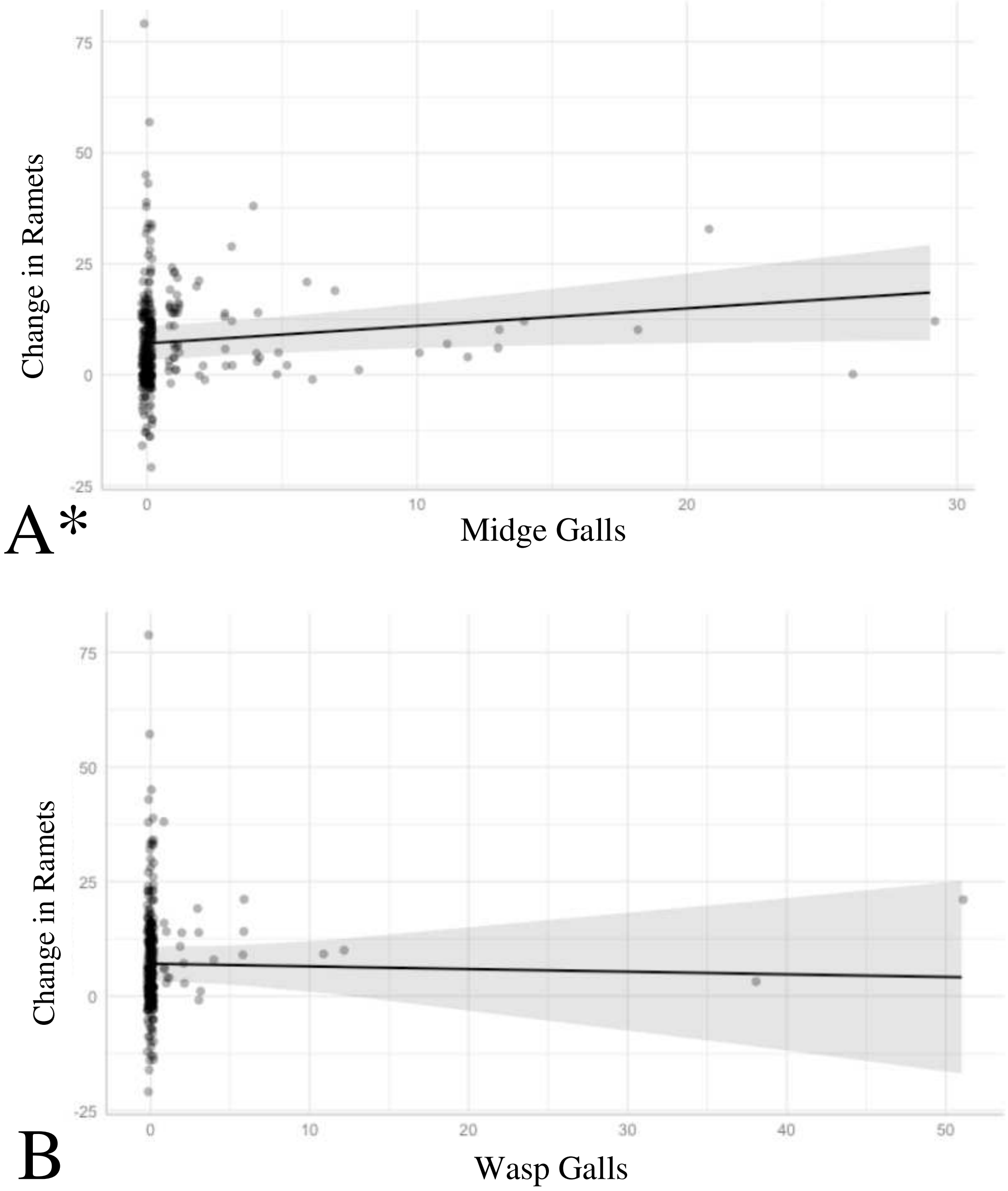

Figure 12. Plots of model-based predictions of the change in the number of ramets with respect to the number of galls of both species (line with standard error shaded in gray) with data (points). The change in the number of ramets was calculated as the initial number of ramets per sampling frame subtracted from the final number of ramets per sampling frame. The scale is on the level of the sampling frame across all 15 sites, with 21 sampling frames per site (N = 315). Marginal R^2 = 0.02, Conditional R^2 = 0.43. (A) Predictions of the change in the total number of ramets based on midge galls (ß = 0.39, p = 0.03, 95% CI: 0.04, 0.75). (B) Predictions of the change in the total number of ramets based on wasp galls (ß = -0.06, p = 0.78, 95% CI: -0.47, 0.35).

Chapter 3 – BIOLOGICAL CONTROL AGENTS DISPLAY POSITIVE AND NEUTRAL INSTRASPECFIC RELATIONSHIPS

INTRODUCTION

When more than one species of insect herbivore attacks a plant, many of the interactions among herbivorous insects are indirect and plant-mediated (Damman et al., 1993; Denno et al., 1995; Kaplan & Denno, 2007; Stephens et al., 2013; Radville et al., 2014; Milbrath & Nechols 2014). A meta-analysis of 243 studies of herbivorous insect interactions found indirect interactions occurring in almost 90% of studies, with changes in plant quality responsible for mediating interactions in over 60% of studies (Kaplan & Denno 2007). Changes in plant quality can be caused by an herbivore altering host plant defenses (Bezemer et al., 2003; Kaplan et al., 2008; Wurst et al., 2008; Erb et al., 2009; Soler et al., 2009; Price et al., 2011; van Dam & Heil, 2011), nutritive content (Inbar et al., 1995; Denno et al., 2000; Johnson et al., 2009), or morphology (Strauss, 1997; Traw & Dawson, 2002; Hall et al., 2012) in such a way that it affects the performance or preference of other herbivores (Ohgushi, 2005; Denno & Kaplan, 2007; Anderson et al., 2011). These plant-mediated effects allow for positive or negative relationships to exist between insects without the individuals being in physical contact or occupying the same space at the same time (Ohgushi, 2005; Denno & Kaplan, 2007; Kaplan & Denno, 2007; Kessler & Halitschke, 2007; Ohgushi, 2008; Ammunét et al., 2010; Anderson et al., 2011; van Dam & Heil, 2011; Cunan, 2015).

Indirect plant-mediated interactions can occur both inter- and intraspecifically, with evidence for both positive (i.e., facilitation and commensalism) (Masters & Brown, 1992; Bronstein, 1994; Masters, 1995; Wallin & Raffa, 2001; Kaplan & Denno, 2007; Ohgushi, 2008;

Bronstein, 2009) and negative (i.e., competition and amensalism) interactions (Bezemer et al., 2003; Kaplan & Denno, 2007; Anderson et al., 2011; Cunan, 2015; Barnes & Murphy, 2018; Castagneyrol et al., 2021). However, in Kaplan and Denno's (2007) meta-analysis of 243 studies, plant-mediated interspecific competition occurred in over 3 times as many observations as facilitation. The nature of these interactions can also be density dependent, with lower insect densities enabling positive or neutral interactions and greater insect densities promoting negative interactions (Milbrath & Nechols, 2014; Pineda et al., 2017). According to classical competition theory, competition should intensify when two organisms co-occur spatially and temporally and are ecologically similar (Kaplan & Denno, 2007). Therefore, intraspecific competition should be stronger than interspecific competition as conspecifics are expected to acquire and consume resources in a more similar manner than heterospecifics (Levins, 1968; Diamond, 1978). However, this assumption may not always apply when considering the prevalence of indirect interactions among phytophagous insects where interactions occur even with spatial and temporal separation and limited niche overlap (Damman, 1993; Kaplan & Denno, 2007). In their meta-analysis on phytophagous insect interactions, Kaplan and Denno (2007) found no significant difference in the strength of competitive interactions between conspecifics and heterospecifics.

Galling insects are excellent organisms with which to study indirect plant-mediated interactions since the insects are confined within the gall and interactions between galls are exclusively restricted to occurring via the plant host. Galls are masses of swollen plant tissue produced in response to oviposition and larval development that provide nourishment and protection to the developing larvae housed within. This nourishment is produced by the plant’s source tissues in the form of photosynthates that the galls draw towards them. In this way, galls function as metabolic sinks that can use the photosynthates for the benefit of the developing larvae housed within. These sinks can compete with other sinks, such as fruits, buds, and meristematic shoot and root tips (Larson & Whitman, 1997), thereby potentially hindering the plant's ability to allocate resources to its own growth and reproduction (Schultz et al., 2013). When multiple insects form galls on the same plant, the interaction of multiple sinks can result in plant-mediated interspecific and intraspecific interactions (Burstein et al., 1994; Inbar et al., 1995; Kaplan et al., 2011; Barosh, 2020).

The system examined in this study consists of the nonnative species Russian knapweed (*Rhaponticum* (=*Acroptilon) repens*) and two gall-forming host-specific insects that are used as biological control agents: the gall midge, *Jaapiella ivannikovi* (Diptera: Cecidomyiidae), and the gall wasp, *Aulacidea acroptilonica* (Hymenoptera: Cynipidae) (USDA APHIS 2008, 2009). These two herbivorous insects and their host plant provide a valuable opportunity to investigate plant-mediated inter- and intraspecific interactions as the insects are confined to their positions within the galls for the overwhelming majority of their lifecycles (Djamankulova et al., 2008; Meyers et al., 2015). Previous studies on the gall midge and gall wasp have indicated that commensalism (+/0) may occur between the insects, wherein wasps benefit from the presence of midges, but midges are neither benefited nor harmed by the presence of wasps (Barosh, unpublished). Barosh et al. (unpublished) found that wasp galls grew larger on stems that had been previously galled by midges, while midge gall size remained unaffected. One explanation for this relationship is that since wasps form galls along the stem, they are able to intercept the flow of photosynthates that is drawn towards the midge galls on the apical meristem of the plant (Figure 13) (Barosh et al., unpublished). Previous studies also indicate that intraspecific competition occurs among midges, with midge galls being wider and producing more larvae when there are fewer midge galls per plant (Barosh, 2020).

Previous research on the impacts of gall midges and wasps on Russian knapweed performance in their native Eurasian range indicates that both insects have dramatic negative impacts on the growth and reproductive capacity of the plant (Djamankulova et al., 2008). Another field study in the introduced range of Colorado found that ungalled ramets grew taller when compared to midge-galled ramets (Barosh, 2020). However, a greenhouse study found that an increase in the number of midge galls was not associated with a decrease in the height or flowering of Russian knapweed, but instead was associated with an increase the aboveground mass, the belowground mass and the number of root buds (Barosh, 2020). This increase in the number of root buds may be due to Russian knapweed compensating for herbivore attack by allocating resources to the production of new ramets (Barosh, 2020). Alternatively, by galling the apical meristem, midges may reduce the production of the plant hormone auxin that establishes apical dominance, resulting in increased lateral branching both aboveground and belowground within the rhizomatic network (Nakamura et al., 2003; Milbrath & Nechols, 2014).

Due to the demand for photosynthates, galls may affect photosynthetic rates in the source tissues of plants (McCrea et al., 1985; Paul & Foyer, 2001; Dorchin et al., 2006). Galls may upregulate the photosynthetic rates of neighboring leaves as a compensatory reaction in plants (Crawley, 1983; Fay et al., 1993; Retuerto et al., 2004; Dorchin et al., 2006; Patankar et al. 2013; Gramig & Harris, 2015). However, several studies have found decreases in photosynthetic rates (Andersen & Mizell, 1987; Larson, 1998; Florentine et al., 2005; Patankar et al., 2011; Huang et al., 2014), and others have demonstrated no significant impact (Larson, 1998). The ability of midge galls and/or wasp galls to affect the photosynthetic rates of Russian knapweed may be a critical mechanism by which the insects are able to alter the plant's growth.

The objectives of this study were to: (1) determine the nature of the interspecific interaction between midges and wasps (i.e. positive, negative, or neutral), (2) determine the nature of the intraspecific interactions among midges and wasps, (3) examine the effects that each insect has on Russian knapweed growth and reproductive capacity and (4) investigate how each insect may affect the photosynthetic rate of Russian knapweed as a possible mechanism by which the insects could alter plant growth. I hypothesized that midges would have a uni-directional positive effect on wasp establishment, indicating a relationship of commensalism (+/0). Therefore, wasps should establish more readily on plants that were previously exposed to midges when compared to plants that were not previously exposed to midges. I further hypothesized that midges would exhibit negative intraspecific interactions. I also predicted that each insect would negatively affect Russian knapweed growth and reproductive capacity, with greater negative effects when both insects established together. Additionally, I hypothesized that both insects would increase the photosynthetic rate of Russian knapweed as a compensatory reaction to galling and/or due to the increased demand for photosynthates. In line with the potentially commensal relationship, I predicted that midges might increase the photosynthetic rate more than wasps, which may be a possible mechanism by which midges can promote wasp establishment.

METHODS

Planting and releasing insects

Eighty-five Russian knapweed rhizome fragments, approximately 2-4cm in length, were gathered from a field site in northern Colorado (N 40°40.473', W 104°58.928') in March 2019. The rhizomes were planted individually in two-gallon pots in the greenhouse. Since they were all collected at a single field site, it is likely that they were from the same individual clone (Gaskin & Littlefield, 2017). Ramets sprouted by early summer, yielding 60 plants that were suitable for attack by the midge and wasp. The 60 ramets were assigned to one of four treatments, with 15 replicates per treatment, in a substitutive 2x2 factorial design to keep the number of ovipositing insects constant (Table 2). Treatments included: 4 midges released, 4 wasps released, 2 midges and 2 wasps released, and a control with no insects released.

All insects were received from the Palisade Insectary of the Colorado Department of Agriculture (Palisade, CO). Wasps were received as adults while midges were received while they were still within their galls. Wasps emerge with their eggs fully developed (i.e., are proovigenic) and oviposit regardless of mating (Djamankulova et al., 2008). When the female midges emerged from their galls, they were kept for 24 hours in the same cage with male midges to ensure mating. The insects were then introduced to the potted plants corresponding to the treatment design. Mesh netting was used to create individual cages around each of the 60 potted plants to prevent the insects from escaping. In the midge + wasp treatment cages, two female midges were introduced to the plants and two female wasps were released 1-4 days after the midges. These releases took place from June 10th through June 14th in 2019. The plants were watered as needed, which was typically to saturation about 4-5 days per week. The greenhouse temperature was maintained within a range of 20°C to 26°C, with a 16L:8D photoperiod. The plants were randomly rotated at least twice per week to control for any variation in temperature and ambient moisture at different locations within the greenhouse.

For each of the 60 plants, I measured the number of aboveground ramets, the height of ramets, and the number of buds per ramet. These data were collected twice during summer 2019, on June 10th (before insects were released) and July 8th (approximately one month after insect release). Any changes in these plant growth parameters were calculated by taking the difference in measurements between these dates. The duration of the experiment was chosen to be as long as possible while remaining within the length of the midge lifecycle of approximately four weeks (Meyers et al., 2015) to prevent the midges from emerging and re-galling the plants.

Data collection

To determine how each insect might alter the photosynthetic rate of Russian knapweed leaves, photosynthetic rates on galled and ungalled plants were measured using an infrared gas analyzer (LI-COR 6400XT, Lincoln Nebraska, USA). A light response curve was constructed at ambient CO_2 concentration (400 µmol CO_2 $m^{-2}s^{-1}$) to determine the saturating light intensity, which was found to be ~1000 µmol photons $m^{-2}s^{-1}$. A photosynthetic curve over the course of a clear morning was also constructed, which determined that there were no significant changes in photosynthetic rates from 9:00am through 1:00pm. Both curves were constructed on the plants within the greenhouse to reflect the experimental conditions. All LI-COR 6400XT measurements were taken on clear days from 9:00am to 12:00pm to avoid any possible midday depression in carbon assimilation rates and stomatal conductance (Roessler & Monson, 1985). Since the leaves of Russian knapweed did not fully cover the infrared gas analyzer (IRGA) gasket, the area of each leaf was measured using calipers before being placed in the IRGA.

Measurements of the photosynthetic rate were taken on a subset of the 60 plants from July 8th through July 10th. The subset consisted of a total of 13 plants, 4 with midges established, 4 with wasps established, 2 with both midges and wasps established, and 3 plants with no insects established. For each plant with galls, the photosynthetic rates of leaves in direct proximity to wasp and midge galls were measured as well as the photosynthetic rates of leaves that were at least 5cm away from midge and wasp galls. The leaves measured always included the third leaf (youngest leaf) below the apical meristem, a leaf at the midpoint of the shoot, and the bottommost undamaged leaf (oldest leaf). The third leaf below the apical meristem was chosen because the upper most leaves were too small and delicate to be consistently measured. Measuring these three leaves allowed us to account for leaf age in the measurements. Depending on the location of the galls on a given plant, these three leaves were at times in direct proximity to galls, which was noted. For all measurements, the reference chamber was set at 400 µmol CO_2 $m^{-2}s^{-1}$; leaf fan speed was set to fast; relative humidity was maintained between 62% to 69%; leaf temperature was periodically set to track ambient conditions (20-23°C); and the stomatal ratio was set to 1. Each leaf was given three minutes to adjust to the IRGA settings before measurements were checked for stability and recorded. Measurements of the photosynthetic rate in mol CO_2 m^{-2} s^{-1} were taken ten times at approximately ten second intervals. These ten measurements were averaged for each leaf measured.

From July 12th through July 15th, the plants were unpotted, the belowground growth was carefully rinsed, and the plants were dried in separate paper bags for three days at 63°C. After drying, the dry weights of the above and below ground growth of each plant were measured. To account for the random loss of very fine root fragments during the unpotting and cleaning process, the dry weight of the below ground growth was standardized by trimming all below ground growth to be equal to or greater than 1mm in diameter. The weights of the plants were obtained to the nearest centigram using a Sartorius CPA2202S-DS scale. The galls were dissected before the plants were dried. The data collected on the galling insects included the number of galls by species, gall width, gall height and the number of larvae per gall. The galls were dissected using a Zeiss Stemi 2000-C stereo dissecting scope with a range of magnification from 6.5x to 50x.

Data analysis

By the end of the experiment, two of the plants in the midge-only treatment died, resulting in the sample sizes by treatment: midges only released (n=13), wasps only released (n = 15), midges and wasps released (n=15), and the control with no insects released (n = 15). Midges and wasps failed to either oviposit or successfully form galls in several of the treatment levels, resulting in the following final sample sizes for insects established by the end of the experiment: only midge galls formed (n = 13), only wasp galls formed (n = 7), midge and wasp galls formed (n = 2), and galls formed (n = 36) (note: sample sizes for gall formation may be subsets or combinations of the release treatments; see Table 2 for more details).

Log likelihood ratio tests were used to determine if the number of observations of gall formation of each insect differed significantly from expected values between the treatments where only one insect was released and the treatment where both insects were released. Log likelihood ratio tests were also used to determine if there was a difference in the expected versus observed values for midges and wasps forming galls separately or together within the midge+wasp treatment.

Normality of the data was confirmed using Quantile-Quantile plots, histograms, and the Shapiro-Wilk test; equal variance was confirmed using residuals vs. fitted plots; and Cook's distance was calculated for all models to ensure that there were no points of high influence.

Because only two (of 15) replicates within the midge+wasp treatment had both midge and wasp galls, when analyzing plant traits, photosynthetic rates, and gall and larvae characteristics, the group with midges and wasps established was dropped (Table 2). When the predictor variables were treated categorically as the three remaining groups (i.e., only midge galls established, only wasp galls established, no galls established) one way ANOVA's were used, followed by pairwise comparisons of estimated marginal means with Tukey adjusted p-values when appropriate. To account for unequal sample sizes, type III ANOVA's were used. When necessary, the data were log or cube-root transformed to meet test assumptions. However, if assumptions were still not able to be met after transformations, then non-parametric tests were used. Specifically, the Kruskal-Wallis rank sum test and the Wilcoxon rank sum test were applied with Holm's adjusted p-values.

In all cases when the predictor variables of wasp galls and midge galls were considered numerically, linear models (LMs) were initially attempted. When the response variables consisted of count data (i.e., the change in the number of buds and the change in the number of ramets), generalized linear models (GLMs) with Poisson regressions were used. However, if the data failed to meet model assumptions even after transformations, then non-parametric Spearman's rank correlations were used. Non-parametric correlations agreed with parametric LMs and GLMs in all instances except when the change in flower buds was a response variable. Nagelkerke's R^2 was calculated for GLMs to compare the log likelihood of the chosen model to that of the intercept model and thereby determine the proportion of variance explained (Nagelkerke, 1991).

Plant traits were analyzed with the predictor variables considered as both categorical and numerical. The following plant traits were treated as response variables: the change in the height of the plants, the final height of the plants, the change in the number of buds on the plants, and the change in the number of ramets. The change in the value of plant variables (plant height, number of buds, number of ramets) was calculated as the initial value subtracted from the final value. The initial value was recorded just before the insects were released and the final value was recorded one month later. When considering the impact on height, both the change in height and the final height were treated as response variables to account for any differences in the initial height of the plants. When the final height was treated as a response variable, the initial height was included as a predictor along with the number of galls of both species. However, since the initial number of buds and the initial number of ramets was almost identical across all plants (with zero buds and one ramet per plant), only the change in these plant traits was analyzed without the initial values used as predictors. When the change in the number of buds was considered as a response variable, the change in the number of ramets was included as a predictor along with the number of galls of both species.

To determine if galls affected the rate of photosynthesis, I examined the relationship between the average rate of photosynthesis per plant and each of the following variables in three separate models: the total number of galls per plant, the number of wasp galls per plant, and the number of midge galls per plant.

To determine the nature of the intraspecific interaction (i.e., positive, negative, or neutral) for each species of insect, the following relationships were considered: the number of galls per plant and the average size of galls per plant, the number of galls per plant and the average number of insects in the galls per plant, and the width of galls and the number of larvae. Since some midges emerged before the end of the experiment, a measurement for the estimated number of insects within a gall was used in addition to the number of larvae. The estimated number of midges included the number of larvae, the number of pupal casings, and the number of adult insects that were found within a gall. When considering similar relationship at the level of each individual gall (i.e., not averaged over the whole plant), the following relationships were also analyzed: the number of larvae and gall size and the estimated number of midges and gall size. Log likelihood ratio tests were also used to determine if there was a difference in the expected versus observed values for each insect species forming only one gall per galled plant or multiple galls per galled plant.

Analyses were conducted using R (R Core Team, 2021) and the R packages lme4 (Bates et al., 2015), emmeans (Lenth, 2020), fmsb (Nakazawa, 2021), ggeffects (Lüdecke, 2018), and ggplot2 (Wickham, 2016).

RESULTS

Overview of insect establishment and the interspecific relationship

A total of 66 wasp galls formed across seven plants and a total of 27 midge galls formed across 13 plants (Table 2). Midges were 4.5 times (0.91, 22.15) more likely to successfully gall a plant than wasps (9/13 vs. 5/15; likelihood ratio $\chi^2 = 3.67$, d.f. = 1, p = 0.055). There is evidence for a neutral interspecific interaction since each insect species was just as likely to form galls when released alone as with the other species. Midges were equally likely to gall a plant when alone (9/13) as they were when in the presence of wasps (6/15) (likelihood ratio $\chi^2 = 2.44$, d.f. = 1, p = 0.12). Similarly, wasps were equally likely to gall a plant when alone (5/15) as they were in the presence of a midge (4/15) (likelihood ratio $\chi^2 = 0.16$, d.f. = 1, p = 0.69).

Intraspecific relationships

Midges demonstrated a neutral intraspecific relationship as they were just as likely to form multiple galls per plant as single galls (9/15 vs. 11/15; likelihood ratio $\chi^2 = 1.75$, d.f. = 1, p = 0.186). The relationships between the number of midge galls per plant and each of the following variables were not significant: the average width of midge galls per plant (S = 574.17, $\rho = -0.0253$, p = 0.93), the average number of midge larvae in each gall per plant (S = 31.557, $\rho = 0.098$, p = 0.85), and the average number of estimated midges in each gall (number of larvae, pupal casings, and adults) per plant (S = 253.76, $\rho = 0.113$, p = 0.73). When looking at the level of each individual midge gall, the relationship between the number of larvae per gall and midge gall width was not significant (S = 52019, $\rho = 0.0497$, p = 0.69). Midge gall width and the estimated number of midges per gall was significantly and positively correlated (S = 61447, $\rho = 0.355$, p = 0.001) (Figure 14).

There was evidence for a positive intraspecific relationship among wasps as they were much more likely to form multiple galls per galled plant than single galls (9/9 vs. 0/9; likelihood ratio $\chi^2 = 7.68$, d.f. = 1, p = 0.005). However, the relationships between the number of wasp galls per plant and each of the following variables were not significant: the average width of wasp galls per plant (S = 107.74, $\rho = 0.10$, p = 0.79), and the average number of wasp larvae per plant (S = 107.64, $\rho = 0.10$, p = 0.79). Considering similar relationships at the level of each individual wasp gall, the relationship between the number of larvae per gall and wasp gall width was not significant (S = 21083, $\rho = 0.198$, p = 0.15).

Impact of insects on plant traits

Midge galls reduced the growth of Russian knapweed such that midge-attacked ramets grew on average 8cm less than ramets with no insect attack ($F_{2,53} = 2.95$, p = 0.06) (Figure 15).

When the number of midge and wasp galls were considered as predictors, the number of midge galls and the change in height showed a negative but not significant correlation (S = 39304, ρ = -0.21, p = 0.12), and the number of wasp galls and the change in height was not significantly correlated (S = 26062, ρ = 0.20, p = 0.14).

There were significant differences in the change in flower buds between groups, such that midge-galled plants produced the greatest number of buds and wasp-galled plants produced the least number of buds (likelihood ratio $\chi^2_{2,53} = 70.63$, $p < 0.0001$) (Figure 16). Midge-galled plants produced over 2 times as many buds as plants with no galls (z ratio = 7.25, p = < 0.0001) and over 5 times as many buds as wasp-galled plants (z ratio = 6.07, p = < 0.0001) (Figure 16). Plants with no galls had over 2 times as many buds as wasp-galled plants (z ratio = 2.75, p = 0.02) (Figure 16). The parametric GLM that used the number of galls of each insect as predictor variables also showed a negative association between the change in flower buds and the number of wasp galls, such that an increase of one wasp gall would be expected to decrease the number of buds by 4% (ß = -0.04, 95% CI: -0.08, -0.004, p = 0.04) (Figure 17). This same GLM showed no significant relationship between the number of midge galls and the change in the number of flower buds (ß = 0.04, 95% CI: -0.04, 0.11, p = 0.33). However, the parametric GLM was not a good fit for the data, so Spearman's rank correlations were also calculated. These correlations showed the change in flower buds was not significantly correlated with the number of midge galls (S = 23655, ρ = 0.19, p = 0.16) or the number of wasp galls (S = 25410, ρ = 0.13, p = 0.33).

There were no significant differences in the change in the number of ramets based on the insects that established by the end of the experiment (Kruskal-Wallis $\chi^2_{2,53} = 1.89$, $p = 0.39$) (Figure 18). The correlation between the number of midge galls and the change in ramets (S =

27110, ρ = 0.02, p = 0.87) and the correlation between the number of wasp galls and the change in ramets were also not significant (S = 22907, ρ = 0.17, p = 0.20).

There were no significant differences in the belowground weight of the plants ($F_{2,53}$ = 1.48, p = 0.24) (Figure 19) or the above ground weight of the plants (Kruskal-Wallis $\chi^2_{2,53}$ = 3.54, p = 0.17) (Figure 20) based on the insects that established. Belowground weight was not significantly correlated with the number of midge galls (S = 29649, ρ = -0.01, p = 0.92) or the number of wasp galls (S = 35649, ρ = -0.22, p = 0.11). The correlation between the number of midge galls and the aboveground weight was also not significant (S = 29770, ρ = -0.02, p = 0.90). However, greater numbers of wasp galls were correlated with increases in the aboveground weight (S = 21944, ρ = 0.25, p = 0.06) (Figure 21).

Photosynthetic rate

Wasp galls seem to increase the photosynthetic rate of the plant, whereas midge galls seem to have no effect on the photosynthetic rate. There was a significant positive correlation between the number of wasp galls per plant and the average rate of photosynthesis per plant (S = 161.14, ρ = 0.56, p = 0.048) (Figure 22). Similarly, there was a marginally significant positive correlation between the number of total galls (i.e., both midge and wasp galls) on a plant and the average rate of photosynthesis per plant (S = 174.38, ρ = 0.52, p = 0.07) (Figure 23). However, the correlation between the number of midge galls per plant and the average rate of photosynthesis per plant was not significant (S = 338.79, ρ = 0.07, p = 0.82) (Figure 24).

DISCUSSION

This study examined the nature (i.e., positive, negative, or neutral) of the interspecific interaction between two phytophagous biological control agents as well as the intraspecific interactions among each insect species. The study further sought to assess what effects each insect has on the growth, reproductive capacity and photosynthetic rates of Russian knapweed. Midges exhibited a neutral intraspecific interaction, which differs from my hypothesis that the intraspecific interaction among midges would be negative. The insects displayed a neutral interspecific interaction, which is contrary to my prediction of a commensal interspecific relationship (+/0) wherein midges would promote wasp establishment. In line with the hypothesized commensal relationship, I predicted that, while both insects would upregulate the rate of photosynthesis, midges would increase the photosynthetic rate more as a potential mechanism that could assist in wasp establishment. However, midges had no impact on the photosynthetic rate (Figure 24), while wasps appeared to upregulate the photosynthetic rate (Figure 22). I further expected that both insects would have negative impacts on Russian knapweed growth and reproductive capacity. This prediction partially aligned with my results as wasps were associated with decreases in the number of flower buds (Figures 16, 17) and midges were associated with decreases in the height of the ramets (Figure 15). However, midges were associated with increases in the number of flower buds (Figure 16) and wasps were associated with increases in the aboveground growth (Figure 21), which contradicts the hypothesized negative impacts of the insects on plant traits.

Interspecific relationship

I did not find any evidence of a significant interaction occurring between midges and wasps, which suggests that the nature of the interspecific interaction may be neutral. Neither insect species was more or less likely to establish based on whether the other insect species was also released. However, only two plants were attacked by both midges and wasps, which limited the assessment of the strength and direction of the interaction and its effect on plant performance. The midges were released only a few days before the wasps, which may not have allowed the midge gall the time necessary to develop into a strong enough sink that could influence wasp growth and establishment. A previous choice test study that allowed the midge galls to more fully develop prior to releasing wasps found that wasps were more likely to oviposit in ramets that were previously galled by midges and that wasp galls grew larger in the presence of midge galls, indicating a commensal relationship (Barosh et al., unpublished). Another study that examined interspecific interactions between a gall midge *Rabdophaga rigidae* (Diptera: Cecidomyaiidae), an aphid, and two species of leaf beetles feeding on willow found that the formation of galls by midges positively impacted the subsequent feeding of the other insect species by causing increases in lateral branching (Nakamura et al., 2003). It seems possible that if the midge galls in this study were given more time to develop before the wasps were released, then the midge galls may have been able to both act as stronger sinks and increase lateral branching that could have provided more galling locations for wasp establishment.

Intraspecific relationships

Wasps were much more likely to form multiple galls than single galls per plant, indicating that a positive intraspecific relationship between wasps may be occurring. This could mean that the ovipositing of one wasp promotes the ovipositing of other wasps and/or that the wasp larvae within the galls more successfully develop when there are multiple galls per plant. The intraspecific relationship among wasps may be more likely to be positive in greenhouse conditions, where resources like water and soil nutrients are not limiting and an abundance of oviposition sites are readily available on plants that have not been extensively galled (Stone et al., 2002). When resources and oviposition sites are limited, intraspecific competition among cynipids can become more likely (Stone et al., 2002).

Midges displayed a neutral intraspecific relationship. This contrasts with a previous study that found evidence for intraspecific competition between midges, with midge galls being less wide and containing fewer larvae when more midge galls were present (Barosh 2020). The study by Barosh (2020) obtained higher densities of midge galls, with a mode of one gall per plant and a maximum of 15 galls per plant. The nature of interactions among herbivorous insects on host plants can be density dependent, with greater densities of insects promoting more negative interactions (Pineda et al., 2017). Therefore, the difference in results may be due to the lower densities of midge galls in the present study when compared to the Barosh (2020) study. Wider midge galls contained more larvae, which suggests that gall width is a useful measure for estimating the number of larvae within a gall, consistent with previous observations (Barosh, 2020).

Russian knapweed growth and reproductive capacity

Midge galls reduced the growth of Russian knapweed such that ungalled ramets were on average 8cm taller than midge-galled ramets (Figure 15). This indicates that midge galls are successfully diverting resources away from plant growth and/or stopping meristem development and elongation. This corresponds with a former study that shows a 12% decline in height due to midge establishment (Djamankulova et al., 2008).

Midges did not impact the aboveground or belowground mass, which contrasts with a greenhouse study that found increases in aboveground and belowground mass in midge attacked ramets (Barosh, 2020). The number of wasp galls showed a marginally significant positive correlation with above ground mass (Figure 21). These results contradict a previous field study in the native Eurasian range that found decreases in aboveground mass due to midge and wasp galling (Djamankulova et al., 2008).

There were mixed results for the impact of the insects on flower bud production. The negative association between wasp galls and the change in flower buds shown in the categorical analysis (Figure 16) and the parametric GLM analysis (Figure 17) both support the prediction that wasp galls would reduce flower bud production. This indicates that wasps are successfully inhibiting plant growth by diverting resources towards larval development, which agrees with previous studies (Djamankulova et al., 2008).

However, the prediction that midge galls would also decrease flower bud production was contradicted by the positive association shown in the categorical analysis (Figure 16) and the neutral association shown in the parametric GLM analysis. Previous research has demonstrated that midges are capable of reducing the development of flower buds (Djamankulova et al., 2008). Though Barosh (2020) found that midge galls had no significant impact on flower bud production. Another species of gall midge *Rabdophaga rigidae* (Diptera: Cecidomyaiidae) caused increases in lateral branching in willow after attacking the apical meristem (Nakamura et al., 2003). Similar effects have been observed when the biological control agent *Trichosirocalus horridus* (Coleoptera: Curculionidae) feeds on the apical meristem of musk thistle (Milbrath & Nechols, 2014). The increase in flower buds in the present study following the galling of the apical meristem aligns with these trends. This effect may be caused by the midges inhibiting auxin production when damaging the apical meristem, thereby reducing apical dominance and encouraging the growth of lateral meristems and buds (Nakamura et al., 2003; Milbrath & Nechols, 2014). Since the midges did reduce the height of Russian knapweed in this study, it seems quite possible that damage to the apical meristem did in fact occur.

It is worth noting that Russian knapweed reproduces primarily clonally through its extensive rhizomatic network (Gaskin & Littlefield, 2017). Therefore, while inhibiting flower bud and eventual seed production is important in preventing the plant from spreading to new distant sites, it may be of equal or greater importance for the biological control agents to be able to inhibit clonal growth (Gaskin & Littlefield, 2017).

The mixed impacts on Russian knapweed growth and reproductive capacity in this study may be due to low densities of the insects and the relatively short duration of the experiment. The densities of both insects in their native range where striking results have been observed is much higher, with up to 12 wasp galls and 15 midge galls per ramet (Djamankulova et al., 2008). Whereas a study performed in Wyoming where midges occur at lower densities found no significant impacts on Russian knapweed growth (Meyers et al., 2015). It seems that the insects likely need to act at greater densities and/or over a longer period of time for dramatic effects to be observed.

Photosynthetic rate

The positive relationship between the total number of wasp galls and the average photosynthetic rate per plant suggests that wasps upregulate the rate of photosynthesis by causing a compensatory reaction in the plant and/or a response to the greater demand for photosynthates. Similar patterns of galling insects upregulating photosynthetic rates in plants have been observed in several studies (Crawley, 1983; Fay et al., 1993; Retuerto et al., 2004; Dorchin et al., 2006; Patankar et al., 2013; Gramig & Harris, 2015). However, other studies found no significant effect of galls on the photosynthetic rates of nearby leaves (Larson, 1998), while others demonstrated decreases in the photosynthetic rates (Andersen & Mizell, 1987; Larson, 1998; Florentine et al., 2005; Patankar et al., 2011; Huang et al., 2014). Part of this variation may be explained by the type of plant tissue galled. Galling insects that do not disrupt the plant's vascular system (e.g., cynipid wasps and cecidomyiid flies; Dorchin et al., 2006; Patankar et al., 2013) may cause an increase in photosynthetic rates of nearby leaves, whereas galling insects that damage the vascular system (e.g., the moth *Epiblema strenuana*; Florentine et al., 2005) may decrease the hosts' photosynthetic rates (Dorchin et al., 2006; Gramig & Harris, 2015). Wasp galls may be able to increase the photosynthetic rates of Russian knapweed since they do not appear to severely disrupt its vascular system.

Bagatto et al. (1996) found that plants with multiple galls allocated over 7 times the amount of assimilates to galled tissues when compared to plants that only had one gall. These findings align with our results in that the rate of photosynthesis seems to be dependent not only on the presence of galls but on the number of galls. This upregulating of photosynthesis may be a mechanism by which greater numbers of wasp galls could cause increases in aboveground growth. It may also help explain the potentially positive intraspecific relationship among wasps since greater numbers of wasp galls could increase the production of photosynthates, which would benefit the developing larvae.

The lack of a significant association between midge galls and the photosynthetic rate per plant may be due to the difficulty of measuring the small fragile leaves near the apical meristem that were in direct proximity to midge galls. If these leaves were able to be measured, then an effect might have been observed.

Conclusions and future directions

The potentially neutral relationship between midges and wasps suggests that these two insects may work well together as biological control agents. The positive intraspecific relationship among wasps should also be beneficial for biological control purposes since it should increasingly enable more wasps to establish. When at higher densities and established for longer periods of time, the two species may have an additive effect in diminishing Russian knapweed growth and reproductive capacity. However, the nature of these relationships may change when the insects occur at high densities and at field sites where there are limited resources. In these conditions, the inter- and intraspecific relationships may become more competitive (Stone et al., 2002; Underwood, 2010; Milbrath & Nechols, 2014; Pineda et al., 2017).

Future studies that specifically trace how nutrients are allocated within the plant to the galls of each species would provide much more detailed information that could assist in determining the nature of the intra- and interspecific relationships of these species and their effects on plant growth and reproductive capacity. This could be performed by measuring carbon allocation patterns through an analysis of the total soluble sugars in different plant tissues (Huang et al., 2015; Zorić et al., 2019) or through using stable isotope analysis (Inbar et al., 1995; Newton, 2016), which has not commonly been used in the study of terrestrial insects (Quinby et al., 2020). Uncovering these relationships and their effects more precisely are vital to managing this widespread non-native species and can contribute to understanding indirect plant-mediated interactions more broadly.

TABLES AND FIGURES

Table 2. An overview of insect establishment (the number of galls formed) by treatment. The predictor variable used in the analysis of plant traits was the total amount of insects of a given species that established across treatments (the last column), with the category of "midge & wasp galls formed" excluded due to a small number of observations (n = 2). Considering the number of wasp galls formed per plant across the wasp and midge+wasp treatments (30 plants total), the median was 0 with a range of (0, 21), the mean was 2.2, and the mode was 0. Considering the number of midge galls formed per plant across the midge and midge+wasp treatments (28 plants total), the median was 1 with a range of (0,6), the mean was 0.96, and the mode was 0.

	Control (n = 15)	Wasp Trt. (n = 15)	Midge Trt. (n = 13)	Midge & Wasp Trt. (n = 15)	Totals Across Trts. (n = 58)
No Galls Formed	15	10	4	7	36
Only Wasp Galls Formed	0	5	0	2	7
Only Midge Galls Formed	0	0	9	4	13
Midge & Wasp Galls Formed	0	0	0	2	2

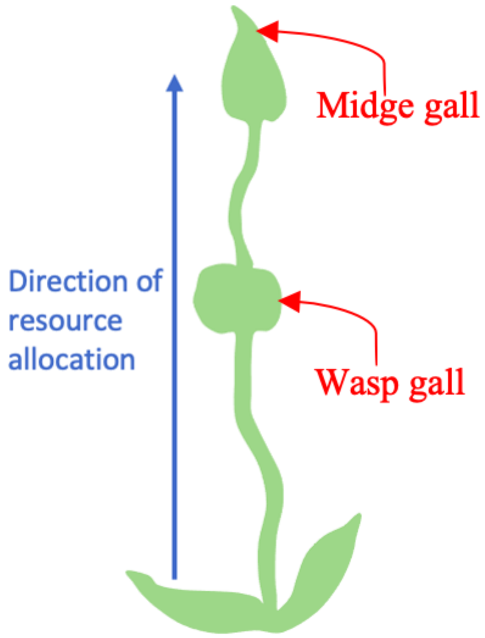

Figure 13. Wasps may benefit from the presence of midge galls if they are able to intercept photosynthates that are drawn towards the midge gall on the apical meristem.

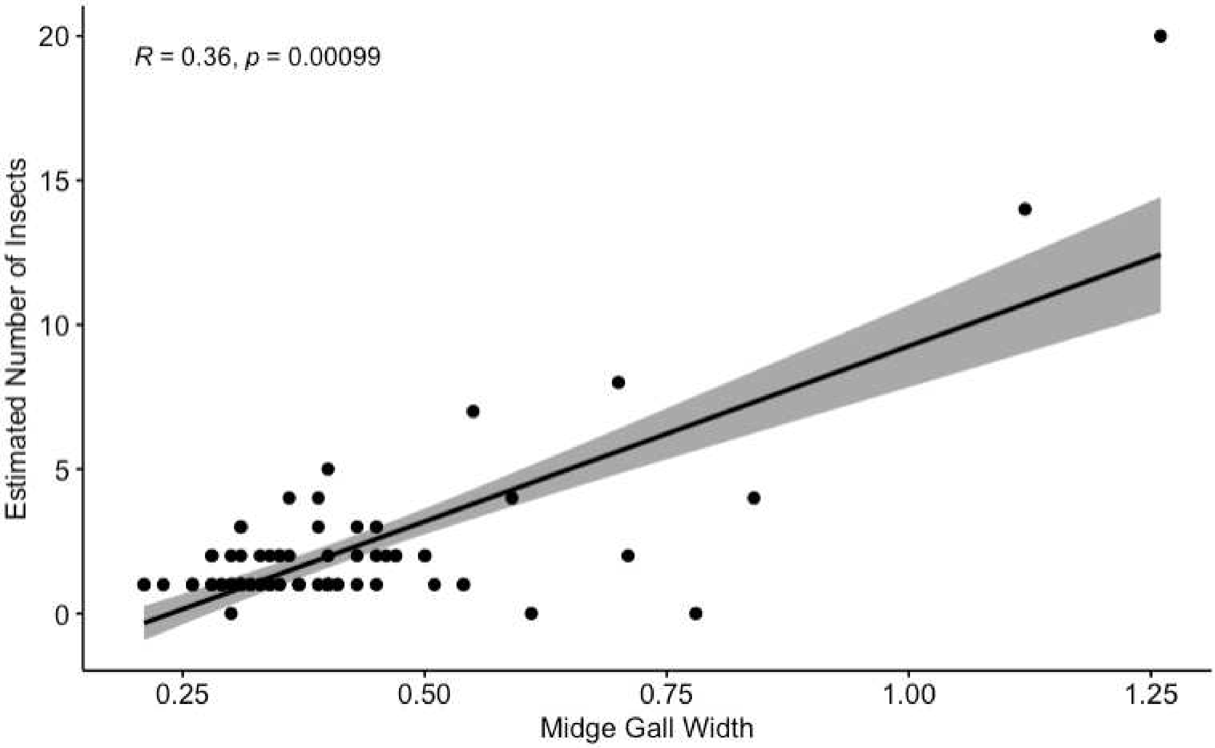

Figure 14. Correlation between midge gall width and the estimated number of midges per gall (S = 61447, ρ = 0.355, p = 0.001). The shaded area represents the 95% confidence interval. This measurement of estimated number of midges includes the number of larvae, the number of pupal casings, and the number of adult insects that were found within a gall.

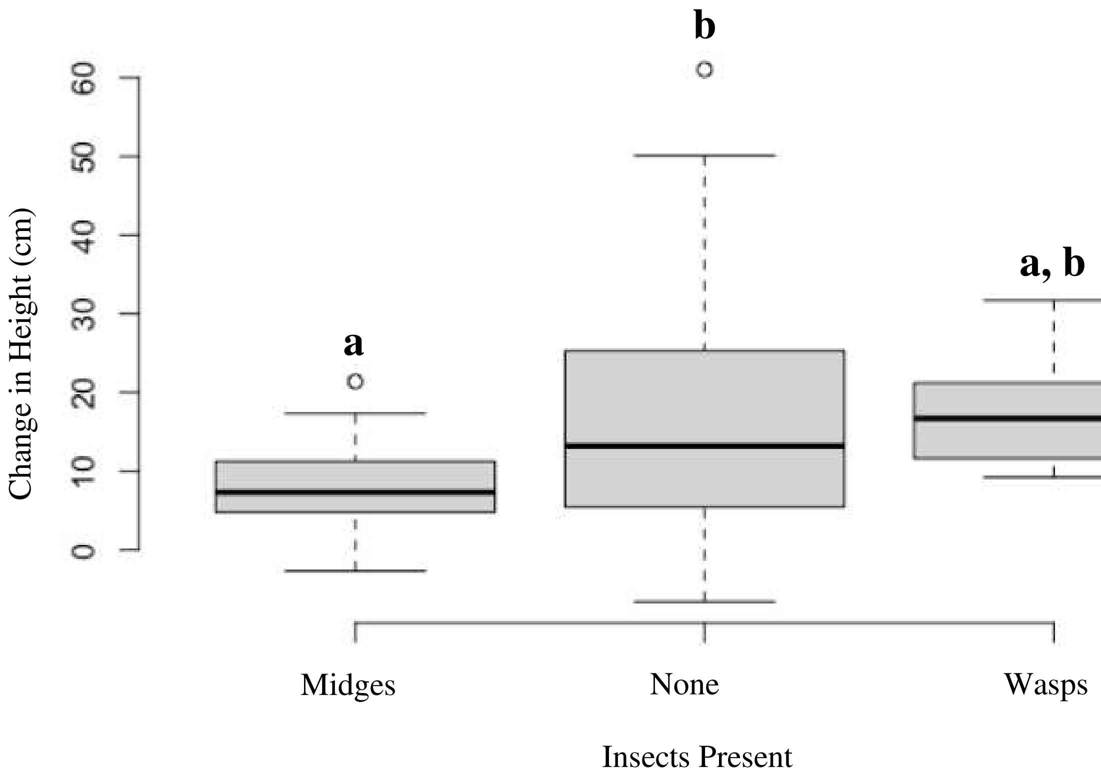

Figure 15. The change in height of the plants over the course of the experiment based on the insects that formed galls. The standard boxplot shows the median as the black midline and the white points as outliers. The statistical difference noted here between the plants with midge galls present and no insects present is for a marginal significant difference in height of an average of 8cm ($F_{2,53} = 2.95$, $p = 0.06$). For plants with midge galls formed: mean (± SE) = 8.5cm (± 1.77cm) For plants where no galls formed: mean (± SE) = 16.5cm (± 2.74cm). For plants with wasp galls formed: mean (± SE) = 17.6cm (± 3.0cm).

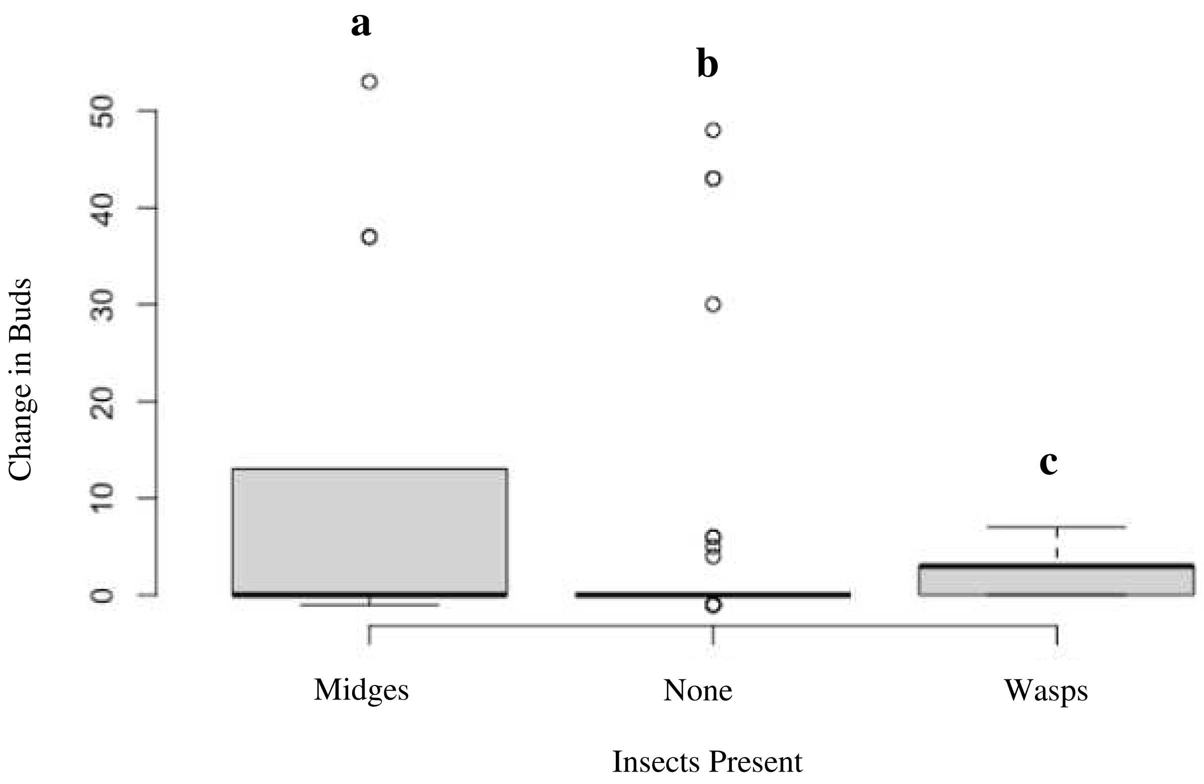

Figure 16. There were significant differences in the change in flower buds between groups with midges only, wasps only, and no insects established (likelihood ratio $\chi^2_{2,53}$ = 70.63, p = 4.6e-16). The standard boxplot shows the median as the black midline and the white points as outliers. Plants with midge galls had 2.34 times (± 0.22) as many buds as plants with no galls (z ratio = 7.25, p = < 0.0001) and 5.12 times (± 0.86) as many buds as plants with wasp galls (z ratio = 6.07, p = < 0.0001). Plants that had no galls had 2.19 times (± 0.40) as many buds as plants with wasp galls (z ratio = 2.75, p = 0.02). For plants with midge galls formed: mean (± SE) = 11.69 (± 5.09). For plants with no galls formed: mean (± SE) = 5.00 (± 2.20). For plants with wasp galls formed: mean (± SE) = 2.29 (± 0.97).

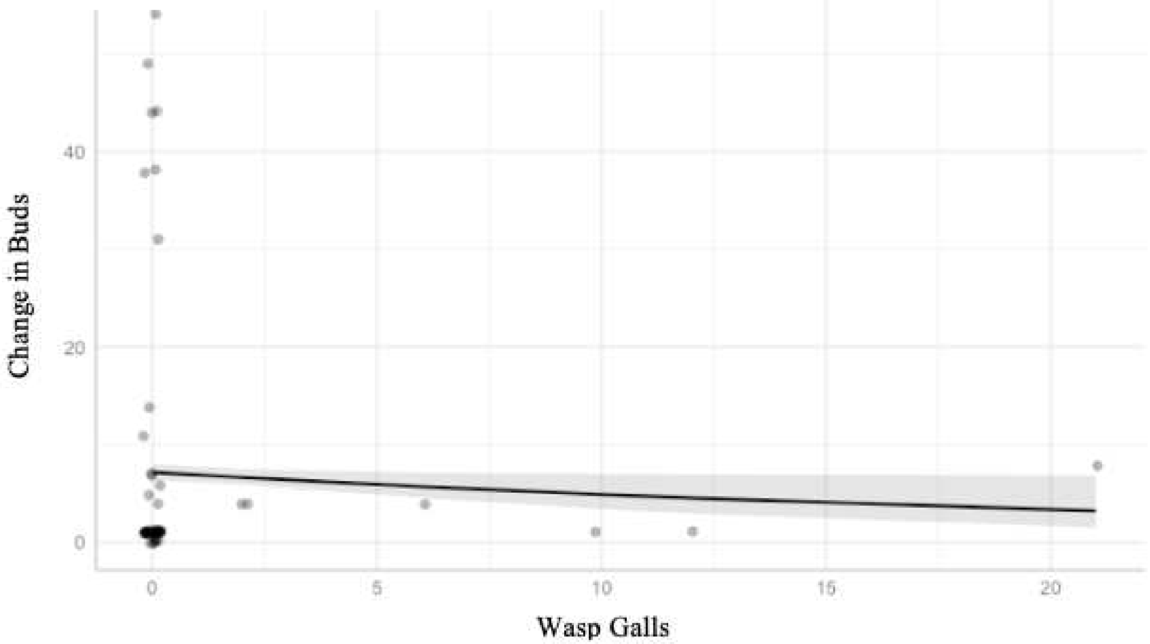

Figure 17. Plot of GLM-based predictions of the number of wasp galls with respect to the change in the number of flower buds (line with standard error shaded in gray) with data (points). An increase of one wasp gall would be expected to decrease the number of buds by 4% (ß = -0.04, 95% CI: -0.08, -0.004, p = 0.04). Nagelkerke R^2 = 0.28.

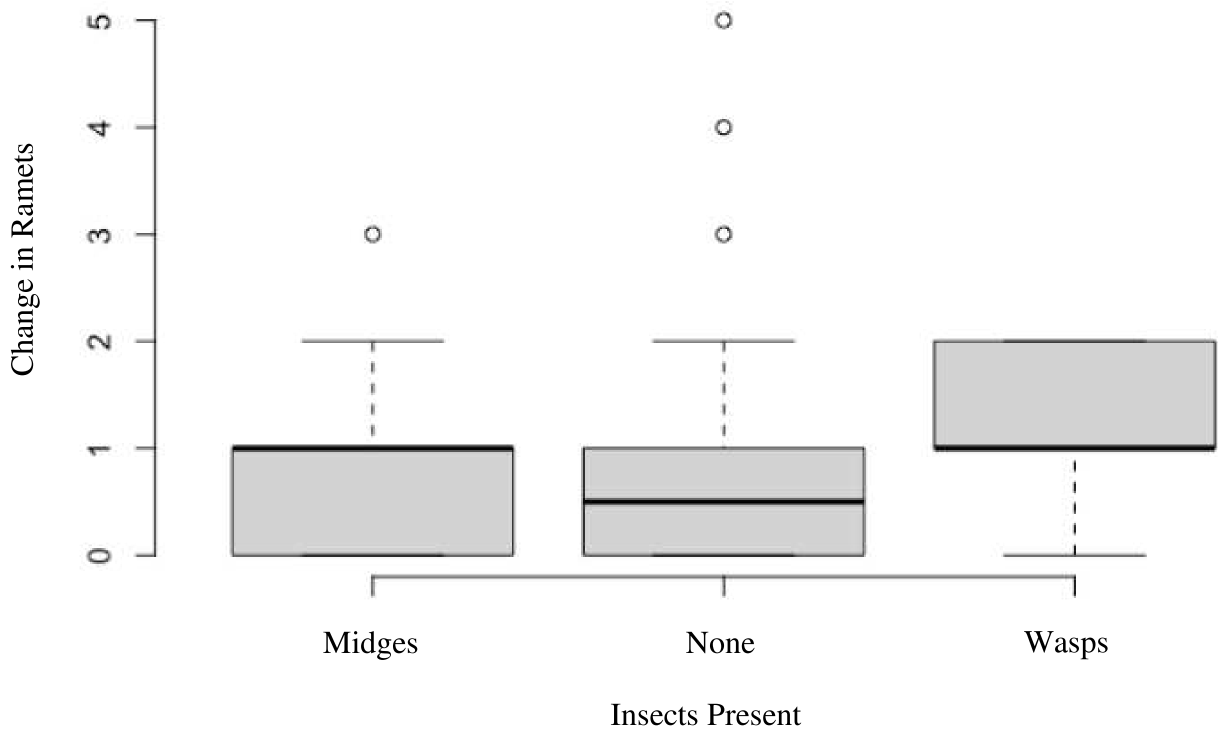

Figure 18. There were no significant differences in the change in the number of ramets based on the insects that established (Kruskal-Wallis $\chi^2_{2,53} = 1.89$, d.f. = 2, $p = 0.39$). The standard boxplot shows the median as the black midline and the white points as outliers. For plants with midge galls formed: mean (± SE) = 0.85 (± 0.27). For plants with no galls formed: mean (± SE) = 0.83 (± 0.20). For plants with wasp galls formed: mean (± SE) = 1.17 (± 0.31).

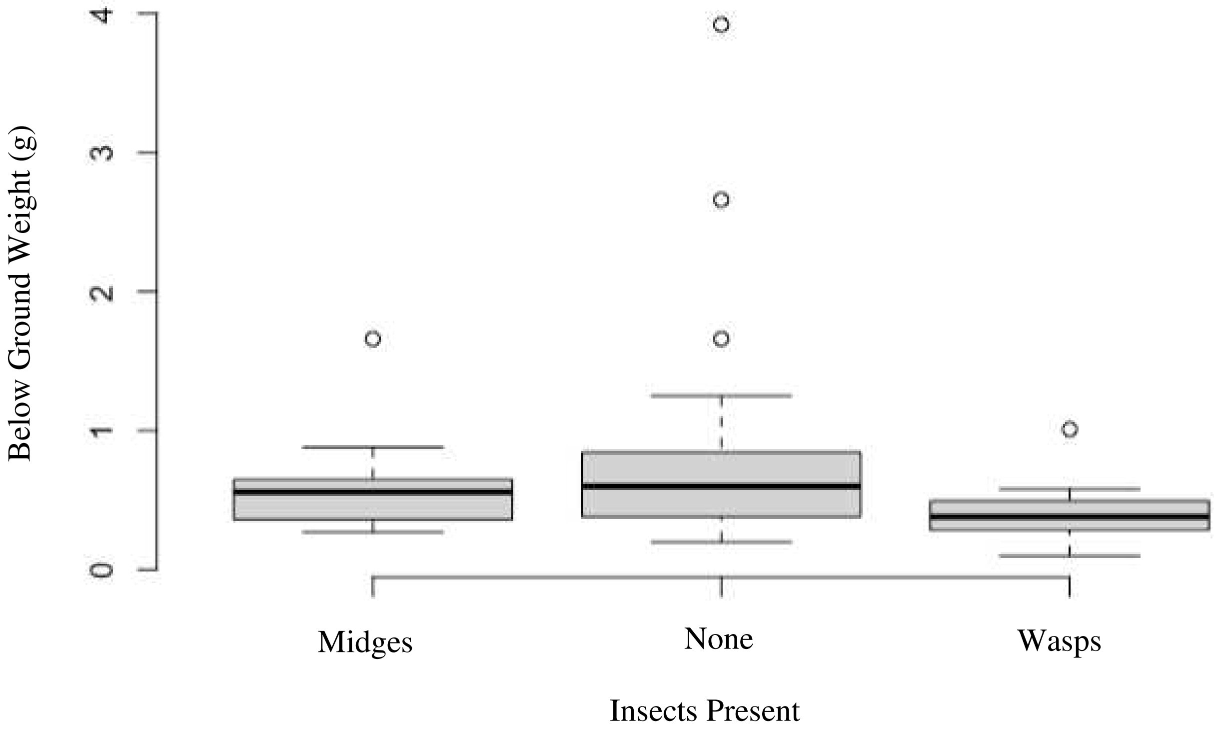

Figure 19. There were no significant differences in the below ground weight of the plants based on the insects that established ($F_{2,53}$ = 1.48, p = 0.24). The standard boxplot shows the median as the black midline and the white points as outliers. For plants with midge galls formed: mean (± SE) = 0.61 (± 0.10). For plants with no galls formed: mean (± SE) = 0.78 (± 0.12). For plants with wasp galls formed: mean (± SE) = 0.44 (± 0.11).

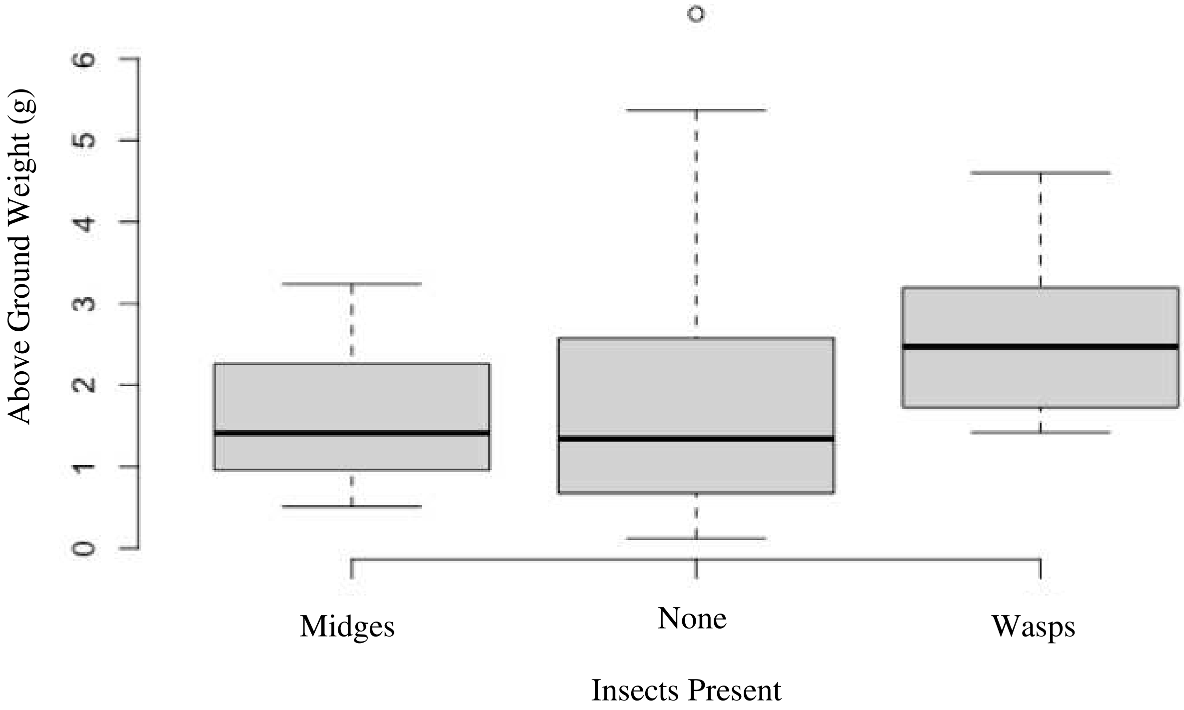

Figure 20. There were no significant differences in the above ground weight of the plants based on the insects that established (Kruskal-Wallis $\chi^2_{2,53} = 3.54$, $p = 0.17$). The standard boxplot shows the median as the black midline and the white points as outliers. For plants with midge galls formed: mean (± SE) = 1.58 (± 0.23). For plants with no galls formed: mean (± SE) = 1.91 (±0.29). For plants with wasp galls formed: mean (± SE) = 2.62 (±0.43).

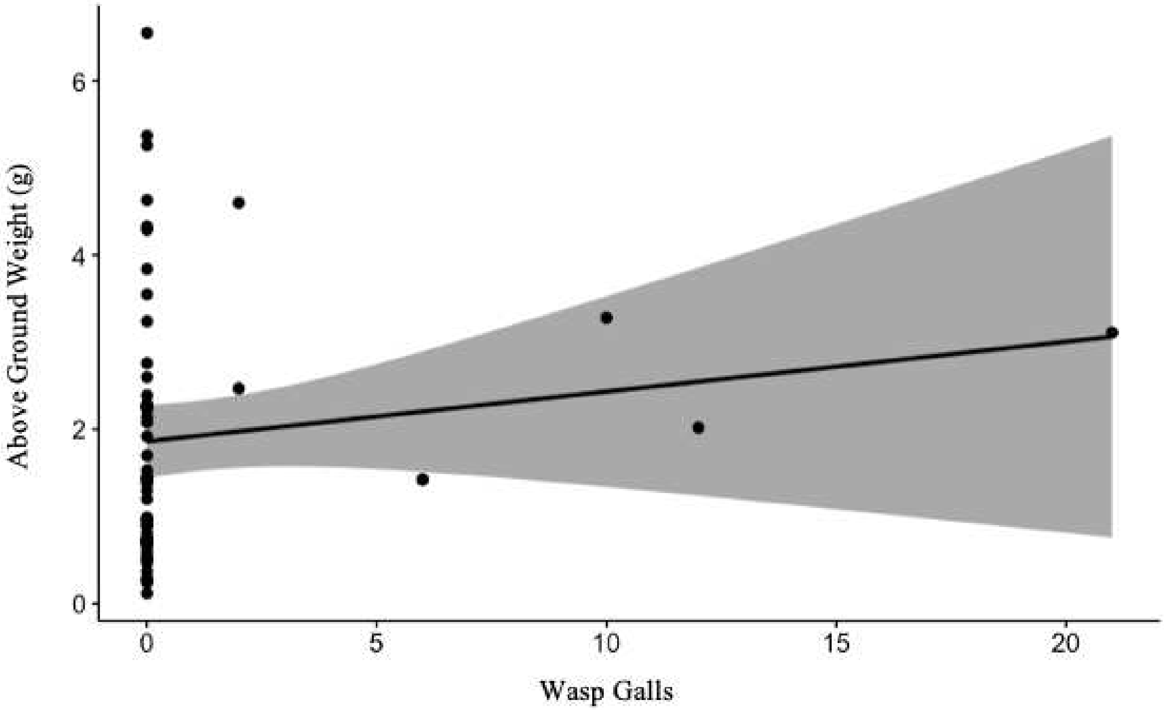

Figure 21. Correlation between the number of wasp galls and the above ground weight (S = 21944, p = 0.06, ρ = 0.25). The shaded area represents the 95% confidence interval.

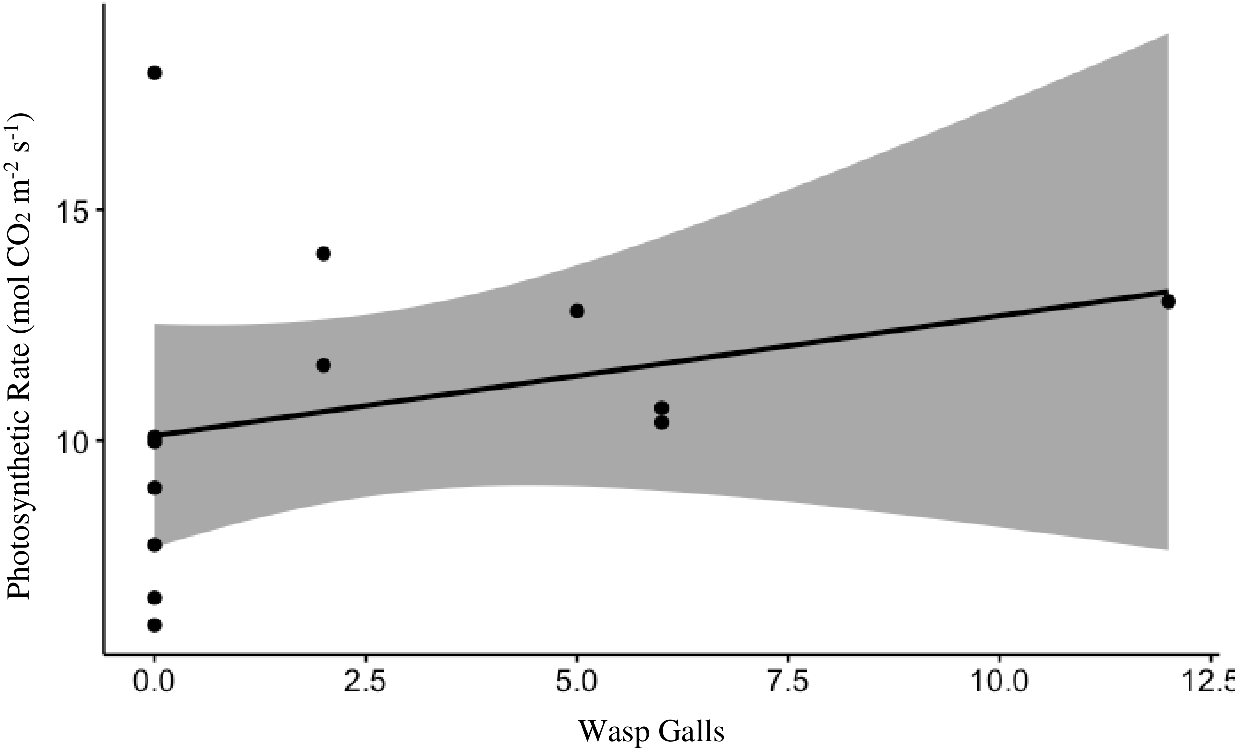

Figure 22. There was a significant correlation between the number of wasp galls per plant and the average rate of photosynthesis per plant (S = 161.14, $\rho = 0.56$, $p = 0.048$). The shaded area represents the 95% confidence interval.

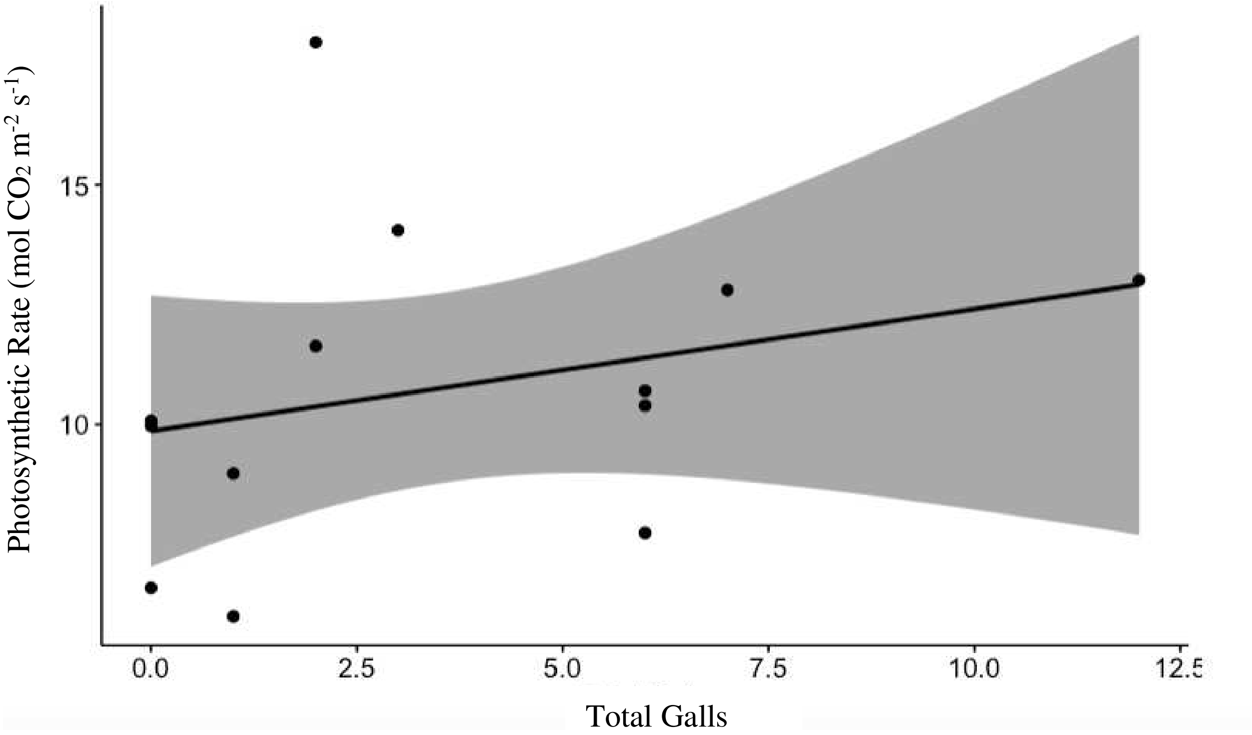

Figure 23. There was a marginally significant correlation between the number of total galls on a plant and the average rate of photosynthesis per plant (S = 174.38, ρ = 0.52, p = 0.07). The shaded area represents the 95% confidence interval.

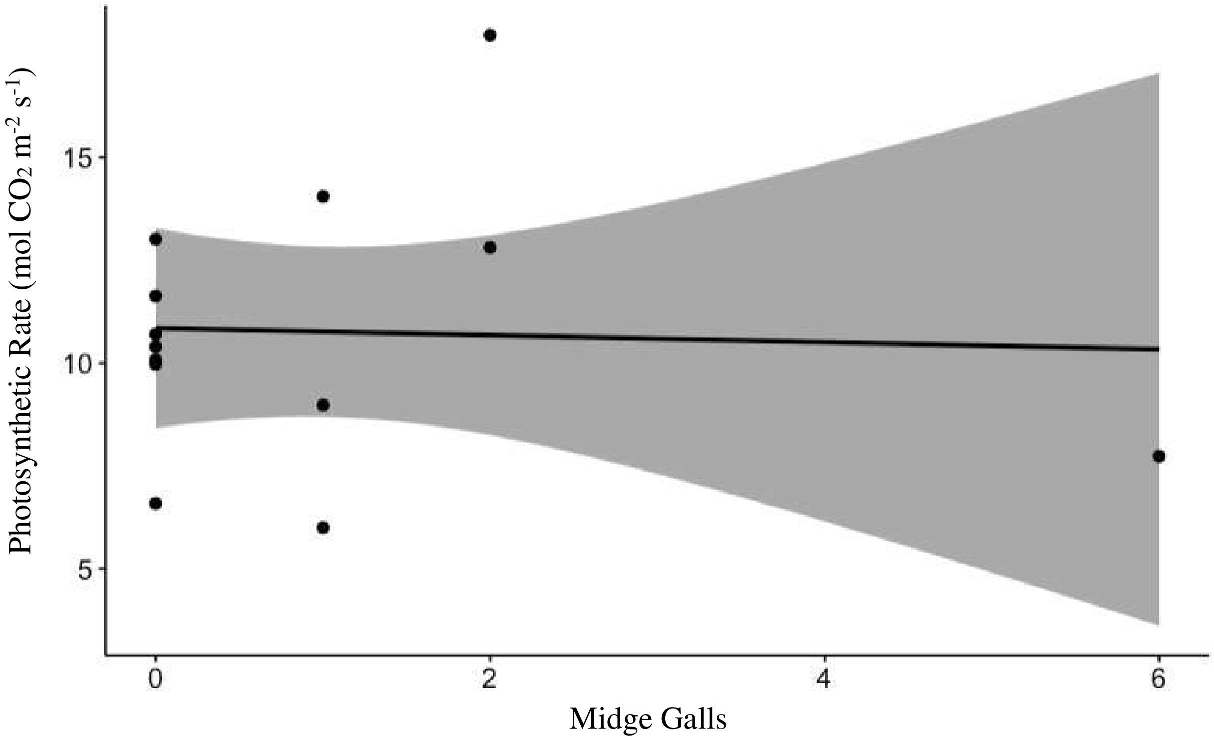

Figure 24. The correlation between the number of midge galls per plant and the average rate of photosynthesis per plant was not significant ($S = 338.79$, $\rho = 0.07$, $p = 0.82$). The shaded area represents the 95% confidence interval.

REFERENCES

Ammunét, T., Heisswolf, A., Klemola, N., & Klemola, T. (2010). Expansion of the winter moth outbreak range: no restrictive effects of competition with the resident autumnal moth. *Ecological Entomology*, *35*(1), 45-52.

Andersen, P. C., & Mizell III, R. F. (1987). Physiological effects of galls induced by *Phylloxera notabilis* (Homoptera: Phylloxeridae) on pecan foliage. *Environmental Entomology*, *16*(1), 264-268.

Anderson, P., Sadek, M. M., & Wäckers, F. L. (2011). Root herbivory affects oviposition and feeding behavior of a foliar herbivore. *Behavioral Ecology*, *22*(6), 1272-1277.

Bagatto, G., Paquette, L. C., & Shorthouse, J. D. (1996). Influence of galls of Phanacis taraxaci on carbon partitioning within common dandelion, Taraxacum officinale. *Entomologia Experimentalis et Applicata*, *79*(1), 111-117.

Barnes, E. E., & Murphy, S. M. (2018). Time-lagged intraspecific competition in temporally separated cohorts of a generalist insect. *Oecologia*, *186*(3), 711-718.

Barosh, T. (2020). *Plant-mediated interactions among gall forming insects* (Doctoral dissertation, Colorado State University. Libraries).

Bartoń, K. (2020). MuMIn: Multi-Model Inference. R package version 1.43.17. https://CRAN.R-project.org/package=MuMIn

Bates, D., Maechler, M., Bolker, B., Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software, 67*(1), 1-48. doi:10.18637/jss.v067.i01.

Beck, K. G. (2003). *Russian knapweed* (Doctoral dissertation, Colorado State University. Libraries).

Bezemer, T. M., Wagenaar, R., Van Dam, N. M., & Wäckers, F. L. (2003). Interactions between above-and belowground insect herbivores as mediated by the plant defense system. *Oikos*, *101*(3), 555-562.

Bronstein, J. L. (1994). Our current understanding of mutualism. *The Quarterly Review of Biology*, *69*(1), 31-51.

Bronstein, J. L. (2009). The evolution of facilitation and mutualism. *Journal of Ecology*, *97*(6), 1160-1170.

Burstein, M., Wool, D., & Eshel, A. (1994). Sink strength and clone size of sympatric, gall forming aphids. *European Journal of Entomology*, *91*(1), 57-61.

Callaway, R. M., Schaffner, U., Thelen, G. C., Khamraev, A., Juginisov, T., & Maron, J. L. (2012). Impact of *Acroptilon repens* on co-occurring native plants is greater in the invader's non-native range. *Biological Invasions*, *14*(6), 1143-1155.

Castagneyrol, B., van Halder, I., Kadiri, Y., Schille, L., & Jactel, H. (2021). Host-mediated, cross-generational intraspecific competition in a herbivore species. *bioRxiv*, 2020-07.

Connell, J.H. (1980). Diversity and the coevolution of competitors, or the ghost of competition past. *Oikos, 35*, 131–138.

Crawley, M. J. (1983). *Herbivory. The dynamics of animal-plant interactions*. Blackwell Scientific Publications.

Cunan, E. T. (2015). Evidence for plant-mediated competition between defoliating and gall-forming specialists attacking *Solidago altissima*. *The American Midland Naturalist*, *173*(2), 208-217.

Damman, H. (1993). Patterns of interaction among herbivore species. *Caterpillars: ecological and evolutionary constraints on foraging.*, 132-169.

De Moraes, C. M., Cortesero, A. M., Stapel, J. O., & Lewis, W. J. (1999). Intrinsic and extrinsic competitive interactions between two larval parasitoids of *Heliothis virescens*. *Ecological Entomology*, *24*(4), 402-410.

Denno, R. F., & Kaplan, I. (2007). Plant-mediated interactions in herbivorous insects: mechanisms, symmetry, and challenging the paradigms of competition past. *Ecological communities: plant mediation in indirect interaction webs*, 19-50.

Denno, R. F., McClure, M. S., & Ott, J. R. (1995). Interspecific interactions in phytophagous insects: competition reexamined and resurrected. *Annual review of entomology*, *40*(1), 297 331.

Denno, R. F., Peterson, M. A., Gratton, C., Cheng, J., Langellotto, G. A., Huberty, A. F., & Finke, D. L. (2000). Feeding-induced changes in plant quality mediate interspecific competition between sap-feeding herbivores. *Ecology*, *81*(7), 1814-1827.

Denoth, M., Frid, L., & Myers, J. H. (2002). Multiple agents in biological control: improving the odds?. *Biological control*, *24*(1), 20-30.

Diamond, J. M. (1978). Niche shifts and the rediscovery of interspecific competition: why did field biologists so long overlook the widespread evidence for interspecific competition that had already impressed Darwin?. *American scientist*, *66*(3), 322-331.

DiTomaso, J. M. (2000). Invasive weeds in rangelands: species, impacts, and management. *Weed science*, *48*(2), 255-265.

Djamankulova, G., Khamraev, A., & Schaffner, U. (2008). Impact of two shoot-galling biological control candidates on Russian knapweed, *Acroptilon repens*. *Biological Control*, *46*(2), 101-106.

Dorchin, N., Cramer, M. D., & Hoffmann, J. H. (2006). Photosynthesis and sink activity of wasp-induced galls in *Acacia pycnantha*. *Ecology*, *87*(7), 1781-1791.

Duncan, C. A., Jachetta, J. J., Brown, M. L., Carrithers, V. F., Clark, J. K., DiTomaso, J. M., ... & Rice, P. M. (2004). Assessing the Economic, Environmental, and Societal Losses from Invasive Plants on Rangeland and Wildlands. *Weed Technology*, *18*(sp1), 1411-1416.

Ehler, L. E., & Hall, R. W. (1982). Evidence for competitive exclusion of introduced natural enemies in biological control. *Environmental Entomology*, *11*(1), 1-4.

Erb, M., Flors, V., Karlen, D., De Lange, E., Planchamp, C., D'Alessandro, M., ... & Ton, J. (2009). Signal signature of aboveground-induced resistance upon belowground herbivory in maize. *The Plant Journal*, *59*(2), 292-302.

Fay, P. A., Hartnett, D. C., & Knapp, A. K. (1993). Increased photosynthesis and water potentials in *Silphium integrifolium* galled by cynipid wasps. *Oecologia*, *93*(1), 114-120.

Florentine, S. K., Raman, A., & Dhileepan, K. (2005). Effects of gall induction by *Epiblema strenuana* on gas exchange, nutrients, and energetics in *Parthenium hysterophorus*. *BioControl*, *50*(5), 787-801.

Gaskin, J. F., & Littlefield, J. L. (2017). Invasive Russian knapweed (*Acroptilon repens*) creates large patches almost entirely by rhizomic growth. *Invasive Plant Science and Management*, *10*(2), 119-124.

Gramig, G. G., & Harris, M. O. (2015). Plant photosynthetic responses during insect effector triggered plant susceptibility and immunity. *Environmental entomology*, *44*(3), 601-609.

Hall, D. R., Amarawardana, L., Cross, J. V., Francke, W., Boddum, T., & Hillbur, Y. (2012). The chemical ecology of cecidomyiid midges (Diptera: Cecidomyiidae). *Journal of*

Chemical Ecology, *38*(1), 2-22.

Hardin, G. (1960). The competitive exclusion principle. *Science*, *131*(3409), 1292-1297.

Harris, P. (1981). Stress as a strategy in the biological control of weeds. In *Beltsville symposia in agricultural research* (Vol. 5, pp. 333-340).

Harris, P. (1991). Invitation paper (CP Alexander fund): classical biocontrol of weeds: its definition, selection of effective agents, and administrative–political problems. *The Canadian Entomologist*, *123*(4), 827-849.

Hatami Hampa, A., Javanmard, A., Alebrahim, M. T., & Sofalian, O. (2018). Allelopathic Effects of Aqueous Extracts from Sorghum (*Sorghum bicolor L.)* and Russian Knapweed (*Acroptilon repens L.*) on Seedling Growth and Enzymes Activity of Wheat, Sugar beet, Common Lambsquarters and Redroot Pigweed. *Journal of Plant Protection*, *32*(1), 101 119.

Hirsch, S. A., & Leitch, J. A. (1996). *The impact of knapweed on Montana's economy* (No. 1189 2016-94257).

Huang, M., H. Chou, Y. Chang, and C. Yang. (2014). The number of cecidomyiid insect galls affects the photosynthesis of *Machilus thunbergii* host leaves. *J. Asia-Pac. Entomol. 17*: 151–154.

Huang, M. Y., Huang, W. D., Chou, H. M., Chen, C. C., Chen, P. J., Chang, Y. T., & Yang, C.M. (2015). Structural, biochemical, and physiological characterization of photosynthesis in leaf-derived cup-shaped galls on *Litsea acuminata*. *BMC plant biology*, *15*(1), 1-12.

Inbar, M., Eshel, A., & Wool, D. (1995). Interspecific competition among phloem-feeding insects mediated by induced host-plant sinks. *Ecology*, *76*(5), 1506-1515.

Jacobs, J., & Denny, K. (2006). *Ecology and management of Russian knapweed (Acroptilon Repens (L.) DC)*. US Department of Agriculture, Natural Resources Conservation Service.

James, R. R., McEvoy, P. B., & Cox, C. S. (1992). Combining the cinnabar moth (*Tyria jacobaeae)* and the ragwort flea beetle (*Longitarsus jacobaeae*) for control of ragwort (*Senecio jacobaea*): an experimental analysis. *Journal of Applied Ecology*, 589-596.

Johnson, S. N., Hawes, C., & Karley, A. J. (2009). Reappraising the role of plant nutrients as mediators of interactions between root-and foliar-feeding insects. *Functional Ecology*, *23*(4), 699-706.

Kaplan, I., & Denno, R. F. (2007). Interspecific interactions in phytophagous insects revisited: a quantitative assessment of competition theory. *Ecology letters*, *10*(10), 977-994.

Kaplan, I., Sardanelli, S., Rehill, B. J., & Denno, R. F. (2011). Toward a mechanistic understanding of competition in vascular-feeding herbivores: an empirical test of the sink competition hypothesis. *Oecologia, 166*, 627-636.

Karban, R. (1989). Community organization of *Erigeron glaucus* folivores: effects of competition, predation, and host plant. *Ecology*, *70*(4), 1028-1039.

Kessler, A., & Halitschke, R. (2007). Specificity and complexity: the impact of herbivore induced plant responses on arthropod community structure. *Current opinion in plant biology*, *10*(4), 409-414.

Levins, R. (1968). Evolution in Changing Environments: Some Theoretical Explorations (No. 2). Princeton: Princeton University Press.

Larson, K. C. (1998). The impact of two gall-forming arthropods on the photosynthetic rates of their hosts. *Oecologia*, *115*(1-2), 161-166.

Larson, K. C., & Whitham, T. G. (1997). Competition between gall aphids and natural plant sinks: plant architecture affects resistance to galling. *Oecologia*, *109*(4), 575-582.

Lenth, R. (2020). emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.4.7. https://CRAN.R-project.org/package=emmeans

Leveque, L., Monge, J. P., Rojas-Rousse, D., Van Alebeek, F., & Huignard, J. (1993). Analysis of multiparasitism by *Eupelmus vuilleti* (Craw)(Eupelmidae) and *Dinarmus basalis* (Rond)(Pteromalidae) in the presence of one of their common hosts, *Bruchidius atrolineatus* (Pic)(Coleoptera Bruchidae). *Oecologia*, *94*(2), 272-277.

Lüdecke, D. (2018). "ggeffects: Tidy Data Frames of Marginal Effects from Regression Models." *Journal of Open Source Software, 3*(26), 772. doi: 10.21105/joss.00772 (URL: https://doi.org/10.21105/joss.00772).

Masters, G.J. (1995) The impact of root herbivory on aphid performance – Field and laboratory evidence. *Acta Oecologica-International Journal of Ecology, 16*, 135–142.

Masters, G.J. & Brown, V.K. (1992) Plant-mediated interactions between two spatially separated insects. *Functional Ecology, 6*, 175–179.

Masters, G. J., Jones, T. H., & Rogers, M. (2001). Host-plant mediated effects of root herbivory on insect seed predators and their parasitoids. *Oecologia*, *127*(2), 246-250.

Marquis, R.J. & Lill, J.T. (2007). Effects of herbivores as physical ecosystem engineers on plant based trophic interaction webs. In: Ecological Communities: Plant Mediation in Indirect Interaction Webs (eds Ohgushi, T., Craig, T.P. & Price, P.W.). Cambridge University Press, London, pp. 246–274.

McCrea, K. D., Abrahamson, W. G., & Weis, A. E. (1985). Goldenrod ball gall effects on *Solidago altissima*: ^{14}C translocation and growth. *Ecology*, *66*(6), 1902-1907.

McNutt, D. W., & Underwood, N. (2016). Variation in plant-mediated intra-and interspecific interactions among insect herbivores: effects of host genotype. *Ecosphere*, *7*(10), e01520.

Meyers, K., Pieropan, N., & Collier, T. (2015). Monitoring a Gall Midge Population on Russian Knapweed (Acroptilon repens). *Invasive Plant Science and Management*, *8*(4), 409-414.

Milbrath, L. R., & Nechols, J. R. (2004). Indirect effect of early-season infestations of *Trichosirocalus horridus* on R*hinocyllus conicus* (Coleoptera: Curculionidae). *Biological Control*, *30*(1), 95-109.

Milbrath, L. R., & Nechols, J. R. (2014). Plant-mediated interactions: considerations for agent selection in weed biological control programs. *Biological Control*, *72*, 80-90.

Miyamoto Y., & Nakamura, M. (2004). Plant phenology-mediated indirect effects: The gall midge opens the phenological window wider for a leaf beetle. *Entomological Science*, *7*(4), 315-322.

Moon, D. C., Moon, J., & Keagy, A. (2010). Direct and indirect interactions. *Nature Education Knowledge*, *3*(10), 50.

Mouttet, R., Bearez, P., Thomas, C., & Desneux, N. (2011). Phytophagous arthropods and a pathogen sharing a host plant: evidence for indirect plant-mediated interactions. *PLoS One*, *6*(5), e18840.

Morrison, R. G., Lownds, N. K., & Sterling, T. M. (1995). Picloram uptake, translocation, and efficacy in relation to water status of Russian knapweed (*Acroptilon repens*). *Weed Science*, *43*(1), 34-39.

Myers, J.H., 1985. How many insect species are necessary for successful biocontrol of weeds? In: Delfosse, E.S. (Ed.), Proceedings of the 6th International Symposium on the Biological Control of Weeds, Agriculture Canada. Canadian Govt. Printing Office, Ottawa, pp. 77–82.

Myers, J. H., Higgins, C., & Kovacs, E. (1989). How many insect species are necessary for the biological control of insects?. *Environmental Entomology*, *18*(4), 541-547.

Newton, J. (2016). Stable isotopes as tools in ecological research. *eLS*, 1-8.

Nakagawa, S., Johnson, P. C., & Schielzeth, H. (2017). The coefficient of determination R^2 and intra-class correlation coefficient from generalized linear mixed-effects models revisited and expanded. *Journal of the Royal Society Interface*, *14*(134), 20170213.

Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods in ecology and evolution*, *4*(2), 133-142.

Nakamura, M., Miyamoto, Y., & Ohgushi, T. (2003). Gall initiation enhances the availability of food resources for herbivorous insects. *Functional Ecology*, *17*(6), 851-857.

Nakazawa, M. (2021). fmsb: Functions for Medical Statistics Book with some Demographic Data. R package version 0.7.1. https://CRAN.R-project.org/package=fmsb

Nagelkerke, N. J. (1991). A note on a general definition of the coefficient of determination. *Biometrika*, *78*(3), 691-692.

Ohgushi, T. (2005). Indirect interaction webs: herbivore-induced effects through trait change in plants. *Annu. Rev. Ecol. Evol. Syst.*, *36*, 81-105.

Ohgushi, T. (2008). Herbivore-induced indirect interaction webs on terrestrial plants: the importance of non-trophic, indirect, and facilitative interactions. *Entomologia experimentalis et applicata*, *128*(1), 217-229.

Patankar, R., Thomas, S. C., & Smith, S. M. (2011). A gall-inducing arthropod drives declines in canopy tree photosynthesis. *Oecologia*, *167*(3), 701-709.

Patankar, R., Starr, G., Mortazavi, B., Oberbauer, S. F., & Rosenblum, A. (2013). The effects of mite galling on the ecophysiology of two arctic willows. *Arctic, antarctic, and alpine research*, *45*(1), 99-106.

Paul, M. J., & Foyer, C. H. (2001). Sink regulation of photosynthesis. *Journal of experimental botany*, *52*(360), 1383-1400.

Pineda, A. N. A., Soler, R., Pastor, V., Li, Y., & Dicke, M. (2017). Plant-mediated species networks: the modulating role of herbivore density. *Ecological Entomology*, *42*(4), 449-457.

Poelman, E. H., & Dicke, M. (2018). Plant-mediated interactions among insects within a community ecological perspective. *Annual Plant Reviews online*, 309-337.

Poveda, K., Steffan-Dewenter, I., Scheu, S., & Tscharntke, T. (2003). Effects of below-and above-ground herbivores on plant growth, flower visitation and seed set. *Oecologia*, *135*(4), 601-605.

Price, P., Denno, R.F., Eubanks, M.D., Finke, D.L. & Kaplan, I. (2011). Insect Ecology: behavior, populations and communities. Cambridge University Press, Cambridge.

Quinby, B. M., Creighton, J. C., & Flaherty, E. A. (2020). Stable isotope ecology in insects: a review. *Ecological Entomology*, *45*(6), 1231-1246.

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Redman, A. M., & Scriber, J. M. (2000). Competition between the gypsy moth, *Lymantria dispar,* and the northern tiger swallowtail, *Papilio canadensis*: interactions mediated by host plant chemistry, pathogens, and parasitoids. *Oecologia*, *125*(2), 218-228.

Reitz, S. R., & Trumble, J. T. (2002). Competitive displacement among insects and arachnids. *Annual review of entomology*, *47*(1), 435-465.

Radville, L., Gonda-King, L., Gomez, S., Kaplan, I. & Preisser, E.L. (2014). Are exotic herbivores better competitors? A meta-analysis. *Ecology, 95*(1), 30–36.

Retuerto, R., Fernandez-Lema, B., & Obeso, J. R. (2004). Increased photosynthetic performance in holly trees infested by scale insects. *Functional Ecology*, *18*(5), 664-669.

Roessler, P. G., & Monson, R. K. (1985). Midday depression in net photosynthesis and stomatal conductance in Yucca glauca. *Oecologia*, *67*(3), 380-387.

Schultz, J. C., Appel, H. M., Ferrieri, A., & Arnold, T. M. (2013). Flexible resource allocation during plant defense responses. *Frontiers in plant science*, *4*, 324.

Schoener, T. W. (1982). The controversy over interspecific competition: despite spirited criticism, competition continues to occupy a major domain in ecological thought. *American Scientist*, *70*(6), 586-595.

Schoener, T.W. (1993). On the relative importance of direct versus indirect effects in ecological communities. In H. Kawanabe, J.E. Cohen & K. Iwasaki (Eds.), *Mutualism and Community Organization* (pp. 365–415). Oxford University Press.

Soler, R., Schaper, S.V., Bezemer, T.M., Cortesero, A.M., Hoffmeister, T.S., Van der Putten, W.H., Vet, L.E.M. & Harvey, J.A. (2009) Influence of presence and spatial arrangement of belowground insects on host-plant selection of aboveground insects: a field study. *Ecological Entomology, 34*, 339–345.

Stephens, A. E., Srivastava, D. S., & Myers, J. H. (2013). Strength in numbers? Effects of multiple natural enemy species on plant performance. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1760), 20122756.

Stone, G. N., Schönrogge, K., Atkinson, R. J., Bellido, D., & Pujade-Villar, J. (2002). The population biology of oak gall wasps (Hymenoptera: Cynipidae). *Annual review of entomology*, *47*(1), 633-668.

Strauss, S. Y. (1997). Floral characters link herbivores, pollinators, and plant fitness. *Ecology*, *78*(6), 1640-1645.

Traw, M.B., & Dawson, T. E. (2002). Reduced performance of two specialist herbivores (Lepidoptera: Pieridae, Coleoptera: Chrysomelidae) on new leaves of damaged black mustard plants. *Environmental Entomology*, *31*(4), 714-722.

Underwood, N. (2010). Density dependence in insect performance within individual plants: induced resistance to Spodoptera exigua in tomato. *Oikos*, *119*(12), 1993-1999.

USDA APHIS. (2008). Field release of *Aulacidea acroptilonica* (Hymenoptera: Cynipidae), an insect for biological control of Russian knapweed (*Acroptilon repens*), in the continental United States. Environmental Assessment June 2008.

USDA APHIS. (2009). Field release of *Jaapiella ivannikovi* (Diptera: Cecidomyiidae), an insect for biological control of Russian knapweed (*Acroptilon repens*), in the continental United States. Environmental Assessment April 2009.

Utsumi, S., & Ohgushi, T. (2008). Host plant variation in plant-mediated indirect effects: moth boring-induced susceptibility of willows to a specialist leaf beetle. *Ecological Entomology*, *33*(2), 250-260.

van Dam, N. M., & Heil, M. (2011). Multitrophic interactions below and above ground: en route to the next level. *Journal of Ecology*, *99*(1), 77-88.

Vieira, C., & Romero, G. Q. (2013). Ecosystem engineers on plants: indirect facilitation of arthropod communities by leaf-rollers at different scales. *Ecology*, *94*(7), 1510-1518.

Wallin, K. F., & Raffa, K. F. (2001). Effects of folivory on subcortical plant defenses: Can defense theories predict interguild processes?. *Ecology*, *82*(5), 1387-1400.

Walling, L. L. (2000). The myriad plant responses to herbivores. *Journal of plant growth regulation*, *19*(2), 195-216.

Watson, A. K. (1980). The Biology of Canadian Weeds: 43. *Acroptilon (Centaurea) repens (L.)*

DC. *Canadian Journal of Plant Science*, *60*(3), 993-1004.

Wetzel, W. C., Screen, R. M., Li, I., McKenzie, J., Phillips, K. A., Cruz, M., ... & Yang, L. H. (2016). Ecosystem engineering by a gall-forming wasp indirectly suppresses diversity and density of herbivores on oak trees. *Ecology*, *97*(2), 427-438.

Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

Woodburn, T. L. (1996). Interspecific competition between *Rhinocyllus conicus* and *Urophora solstitialis*, two biocontrol agents released in Australia against *Carduus nutans*. In *Proceedings of the 9th international symposium on biological control of weeds, Stellenbosch, South Africa, 19-26 January 1996.* (pp. 409-415). University of Cape Town.

Wootton, J. T. (1994). The nature and consequences of indirect effects in ecological communities. *Annual review of ecology and systematics*, *25*(1), 443-466.

Wootton, J. T. (2002). Indirect effects in complex ecosystems: recent progress and future challenges. *Journal of Sea Research*, *48*(2), 157-172.

Wurst, S., Van Dam, N. M., Monroy, F., Biere, A., & Van der Putten, W. H. (2008). Intraspecific variation in plant defense alters effects of root herbivores on leaf chemistry and aboveground herbivore damage. *Journal of chemical ecology*, *34*(10), 1360-1367.

Zangerl, A. R., & Rutledge, C. E. (1996). The probability of attack and patterns of constitutive and induced defense: a test of optimal defense theory. *The American Naturalist*, *147*(4), 599-608.

Zorić, A. S., Morina, F., Toševski, I., Tosti, T., Jović, J., Krstić, O., & Veljović-Jovanović, S. (2019). Resource allocation in response to herbivory and gall formation in *Linaria vulgaris. Plant Physiology and Biochemistry*, *135*, 224-232.