DISSERTATION

FROM GENES TO LANDSCAPES: DIRECT AND INDIRECT INTERACTIONS ACROSS ECOLOGICAL SCALES

Submitted by

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ABSTRACT

FROM GENES TO LANDSCAPES: DIRECT AND INDIRECT INTERACTIONS ACROSS ECOLOGICAL SCALES

Interactions within and between species profoundly shape ecosystems, influencing community structure, ecosystem function, and evolutionary trajectories. This dissertation examines how direct and indirect interactions vary across spatial, temporal, and ecological scales using a multitrophic plant-herbivore network consisting of the invasive plant Russian knapweed (*Rhaponticum repens*), and its two biological control insects: the gall wasp *Aulacidea acroptilonica* and gall midge *Jaapiella ivannikovi*.

I address three central questions: (I) What mechanisms drive plant-mediated interactions between insect herbivores? (II) Does *R. repens* ecotype selection influence its interactions with herbivores? and (III) How do climate variables and global change influence contemporary and future interactions between specialist plant-insect networks?

To address these questions, I: (I) perform network analysis of plant-insect communities; (II) perform reciprocal transplants and phylogenetic analysis of *R. repens* populations; and (III) examine plant-insect-climate interactions across spatial and temporal scales.

My findings emphasize the interconnectedness of ecological systems, revealing how plant-mediated effects drive indirect interactions between herbivores across ecological scales. Density-dependent interactions and gall community composition emerge as key drivers of herbivore performance, suggesting plant defense activation as the primary mechanism mediating asymmetric interactions between species. *Rhaponticum repens* exhibits evidence for rapid climate driven evolution in its invasive range resulting in dramatically increased resistance to herbivory. Climate modeling reveals complex responses to environmental change, including context-dependent herbivore facilitation under stress and unexpected species niche convergence under severe climate scenarios.

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Chapter 1· Introduction

Background

Interactions within and between species profoundly shape ecological systems, influencing community structure, ecosystem function, and evolutionary trajectories. Direct interactions such as predation, facilitation, and competition have long been recognized as foundational in ecological theory (Hairston, Smith, & Slobodkin 1960; Paine 1966). However, indirect interactions, which occur when the effect of one species on another is mediated by a third species, are increasingly recognized for their importance in structuring ecological systems (Sotomayor & Lortie 2015). In fact, they are thought to outnumber direct interactions in most systems, with ecological communities containing many more weak, indirect interactions than strong, direct interactions (Berlow *et al*. 1999; Aschehoug & Callaway 2015).

Despite having modest effects at the individual level, indirect interactions play a crucial role in stabilizing communities and promoting biological diversity either by opposing direct effects or distributing the impact of strong interactions across many species (Brooker *et al*. 2008; McIntire & Fajardo 2014). Some of the best-documented examples include apparent competition and apparent facilitation, in which two prey species influence one another through a shared predator—altering predation pressure by subsidizing predator populations or diluting individual predation risk (Berlow *et al.* 1999; Aschehoug & Callaway 2015). Other, less frequently studied forms of indirect interactions include exploitative competition and exploitative facilitation, in which organisms interact through changes in the quality or availability of a shared resource (Sotomayor & Lortie 2015; Graff, Rositano, & Aguiar 2013). These interactions are particularly prevalent in plant communities (Sotomayor & Lortie 2015), where herbivores mediate indirect interactions between plants while plants simultaneously mediate indirect interactions between herbivores, resulting in complex feedbacks.

Herbivore-induced changes in plant chemistry, morphology, or physiology can modify plant interactions with subsequent herbivores, pollinators, or pathogens. For example, herbivore-induced plant volatiles (HIPVs) can activate plant defense compounds in distant plant tissues or signal natural enemies of plant herbivores, particularly parasitoids (War *et al*. 2011). These plant-mediated interactions are often specific and highly context-dependent. In one study, the performance of caterpillars of *Mamestra brassicae* feeding on wild cabbage depended on prior herbivory by *Brevicoryne brassicae* aphids and/or *Plutella xylostella* caterpillars, with each species exhibiting unique effects on plant resource quality and defense induction (Kroes *et al*. 2016).

The complexity of these interactions is further magnified by eco-evolutionary feedbacks, in which ecological interactions drive evolutionary changes which in turn influence ecological dynamics (Mooney & Cleland 2001). Climate change illustrates this process, as environmental stressors drive rapid evolutionary changes in species that alter their ecological interactions and community-level dynamics (González-Tokman et al. 2020). These effects may be particularly pronounced in novel or rapidly changing environments. Anthropogenic climate change imposes novel selective pressures that influence both the ecological outcomes and evolutionary trajectories of interacting species. While some species respond through phenotypic plasticity, non-genetic changes in trait expression (Nicotra *et al.* 2010), others exhibit rapid evolution, reshaping the nature and strength of direct and indirect interactions (Yamamichi, Yoshida, & Sasaki 2011; Oostra *et al*. 2018).

Considering the importance of indirect effects in structuring communities and maintaining biodiversity, understanding how indirect interactions are maintained or lost under environmental change is critical for conservation biology. Additionally, because indirect interactions are often fragile and highly context-dependent (Kadoya & McCann 2015), they may be particularly vulnerable to global change (Telfer & Bown 2012; Koch *et al*. 2024). These links may therefore serve as early indicators of community destabilization, informing biodiversity conservation prioritization and management efforts.

To assess how direct and indirect interactions vary across spatial, temporal, and ecological scales, we examine plant-insect interactions across a series of experiments (Figure 1) designed to:

1. Investigate the mechanisms driving plant-mediated indirect interactions between insect herbivores (Ch. 2),
2. Identify how climate-induced evolutionary changes in the host plant influence its interactions with herbivores (Ch. 3), and
3. Describe how global change influences species interactions at the landscape level across mid- and late-century climate scenarios ranging from mild to severe (Ch. 4).

This multi-scale approach allows us to integrate physiological, ecological, and evolutionary processes, linking mechanistic insights to broader ecological and biogeographical patterns and bridging fine-scale experiments with landscape-level predictions.

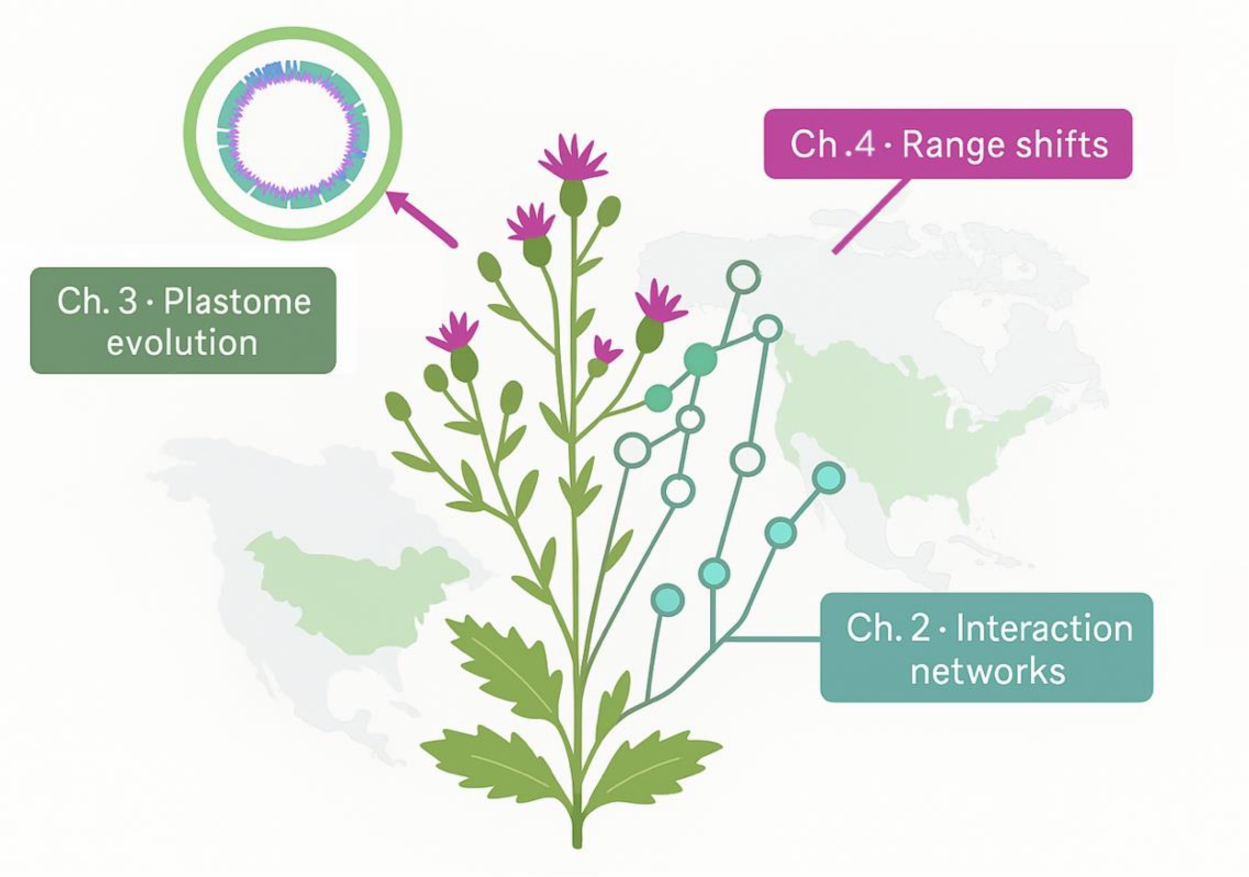


Figure 1. Plant-insect dynamics differ across spatial, temporal, and ecological scales. Interaction networks (Ch. 2) explore plant-mediated insect interactions at the scale of a single plant, evolution from the scale of genomes to plant-herbivore communities (Ch. 3), and the impacts of global change at the ecosystem-level (Ch. 4).

Together these approaches address a central question: how do interactions between species respond to processes operating across broader temporal and spatial scales? Each experiment in Figure 1 represents a different aspect of this question. By first establishing how species interact at the scale of a single plant and then investigating how evolutionary adaptation and climate change influence these interactions, we gain unique insights into how direct and indirect interactions shift across ecological scales, encompassing spatial, temporal, and organizational (e.g., individual, population, landscape) scales.

Study system

To address our research questions, we employ a multitrophic plant-herbivore network consisting of the invasive plant Russian knapweed **(***Rhaponticum repens* (L.) Hidalgo) and its two biological control insects, the gall wasp *Aulacidea acroptilonica* (Hymenoptera: Cynipidae) and the gall midge *Jaapiella ivannikovi* (Diptera: Cecidomyiidae) which act as natural enemies of *R. repens* in its native range. *Rhaponticum repens* was introduced to North America in the late 19th century as a contaminant in alfalfa seed (Djamankulova, Khamraey, & Schaffner 2008). It has since become widespread, displacing native species and degrading rangeland ecosystems across more than 600,000 hectares of land in the U.S. alone in 2000 (Marshall *et al*. 2024). Consequently, the gall wasp and midge*,* were approved for release as classical biological control agents in 2008 and 2009, respectively (USDA 2008, 2009). In Colorado, both agents have been distributed across the state as part of the Colorado Department of Agriculture's biological control program. Our experiments sample populations across knapweed's invasive range in the state (Figure 2).

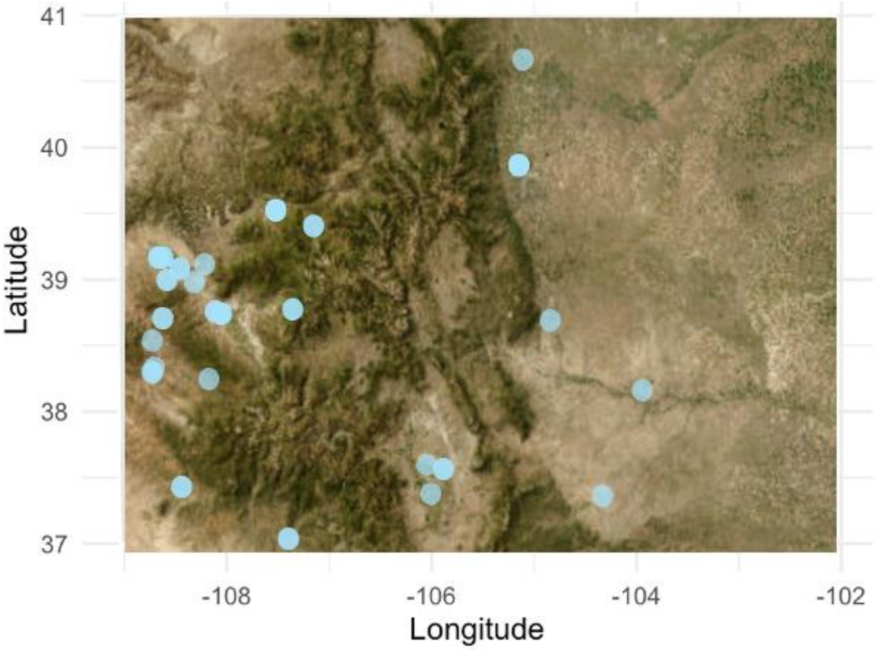


Figure 2. Sampled *R. repens*-biocontrol release sites across the state of Colorado.

The midge and wasp exhibit distinct differences in their life histories and utilization of plant resources. Both insects induce tumor-like nutritive chambers (galls) to form around their offspring which then feed on photosynthates and other plant resources until emerging as adults. The midge *J. ivannikovi* targets nutrient-rich meristematic tissue at the actively growing tips of plants, where it induces the plant to form leafy rosette galls around dozens of eggs deposited on the plant surface (exophytic oviposition). The wasp *A. acroptilonica* exhibits endophytic oviposition, laying its eggs inside plant stems, where each induces the formation of a hard, spherical gall. The wasp deposits a single egg before moving several millimeters up or down the stem to lay another. Additionally, while the wasp is univoltine, with a single generation per year, the midge is multivoltine with approximately six to eight generations throughout the growing season in Colorado. Despite occupying different tissues, both insects rely exclusively on *R. repens*, lacking alternative hosts. This specificity creates an ecologically and evolutionarily interdependent system where interactions between insects are mediated by changes in plant physiology, resources, or induced defenses.

Moreover, the novel environmental conditions experienced in their introduced range raise the potential for strong eco-evolutionary feedbacks between species. The Enemy Release Hypothesis suggests that the success of invasive species is often driven by the absence of natural enemies in their invasive range (Keane & Crawley 2002; Telfer & Bown 2012). The reintroduction of natural enemies after more than a century of geographic separation presents a rare opportunity to examine how plant evolutionary responses to both novel environmental conditions and release from herbivory shape plant-insect dynamics once they are restored.

Thus, the unique dynamics among *R. repens, J. ivannikovi,* and *A. acroptilonica* provide a valuable opportunity to investigate how interactions among coevolved species networks unfold in a novel ecological context. Understanding the mechanisms that govern these interactions not only offers insights into classical ecological and evolutionary theory but also addresses applied challenges in invasive species management and biological control programs.

Chapter 2 · Network analysis reveals complex plant-mediated insect interactions

Overview

Plant-mediated insect interactions are of major theoretical and practical interest, enabling exploration of questions central to classical ecological theory regarding apparent and trait-mediated indirect interactions. Here, we explore how interactions change across domains associated with gall composition, density, and plant architecture. To represent these influences, we constructed quantitative and qualitative interaction networks of two gall-forming biological control agents, a midge *Jaapiella ivannikovi* and a wasp *Aulacidea acroptilonica,* on the invasive plant *R. repens.* We use these networks to examine host plant-mediated interspecific and intraspecific interactions between the two herbivores by observing herbivore gall diameters (a fitness proxy correlated with insect emergence from galls). Our final models predicted distinct species-specific herbivore responses, with herbivores exhibiting functional niche partitioning. Furthermore, models that quantified interactions across multiple network domains and between both species of insect consistently outperformed single-domain or single-species models, emphasizing the interconnectedness of multi-species networks and providing insights into the mechanisms that shape them. Overall, we find evidence of context-dependent, amensalism between herbivores characterized by negative herbivore-induced effects on the host plant which are mediated by gall composition and density at the local and systemic scale.

Introduction

Plant-insect and plant-mediated insect interactions occur in interconnected, multilayered systems where herbivores simultaneously influence and respond to resource quality and quantity, plant defenses, and inter- or intraspecific interactions. Understanding how plant traits mediate interactions between insect herbivores is therefore a central question in ecology. Insects may compete indirectly through changes to plant tissues, such as by inducing plant defenses or via exploitative competition, reducing the quality and/or quantity of plant resources available to other herbivores (Denno & Kaplan 2007). Conversely, they may exhibit indirect facilitation by suppressing host plant defensive compounds (Soler *et al*. 2005; Soler *et al*. 2012) or may demonstrate exploitative facilitation by acting as metabolic sinks that benefit neighboring galls by concentrating plant resources in their vicinity (Kutyniok & Müller 2013).

However, our understanding of indirect interactions is limited by logistical barriers. Experimental approaches to studying plant-mediated insect interactions are particularly challenging, requiring numerous treatment combinations to adequately represent the range of conditions under which insects may interact (Sotomayor & Lortie 2015). They may also necessitate expensive and technically complex techniques to quantify resource flow and defense compounds. This challenge is compounded by the numerous factors that influence plant-insect interactions. For instance, the spatial arrangement of insects on a plant can substantially alter the direction and magnitude of observed interactions, especially for sessile herbivores like galling insects. Thus, new experimental and analytical frameworks are needed to address the challenges of studying these complex interactions.

Quantitative network analysis is particularly well-suited for this task, as it accounts for interdependencies between species while allowing potential mechanisms to be isolated or integrated. It also enables competing and complementary effects to be assessed simultaneously, including across unique sets of connected species. For instance, interactions between individuals may exhibit strong spatial structure while showing only weak effects of species composition, or vice versa. These unique factors can be modeled using multilayer networks*,* which represent distinct relationships among the same set of nodes using separate layers (Figure 3). These qualities make network analysis a powerful tool for identifying system-level patterns and behaviors that arise from complex interactions among multiple components. These emergent properties are particularly important for understanding indirect interactions; for instance, one might find that herbivore-herbivore interactions shift depending on spatial arrangement, time between feeding, or environmental conditions. Quantitative interaction networks have been used successfully to model mating networks of pollinators (Fortuna *et al*. 2008); however, they are overall underutilized in research on indirect interactions in plant-insect networks.

Herbivore life history traits are often associated with distinct plant-mediated interaction mechanisms. For example, gall size and position influence the ability of galls to assimilate plant resources, also known as sink strength, which in turn modifies local plant conditions (Kutyniok & Müller 2013). Gall-forming insects with large, multi-larval galls positioned at nutrient-dense meristematic tissues, such as *J. ivannikovi*, typically function as strong metabolic sinks. Such strong sinks may concentrate resources in their vicinity, potentially benefiting neighboring herbivores through enhanced local resource availability (Tooker *et al*. 2008). Additionally, one method employed by classical biological control insects like the midge to control their invasive hosts is by inducing compensatory growth, which causes plants to divert belowground resources to aboveground tissues to compensate for resources siphoned by galls acting as strong metabolic sinks (Hilbert *et al*. 1981; McNaughton 1983). Although total plant resources are reduced, aboveground resource quality and availability increases, thereby facilitating herbivores.

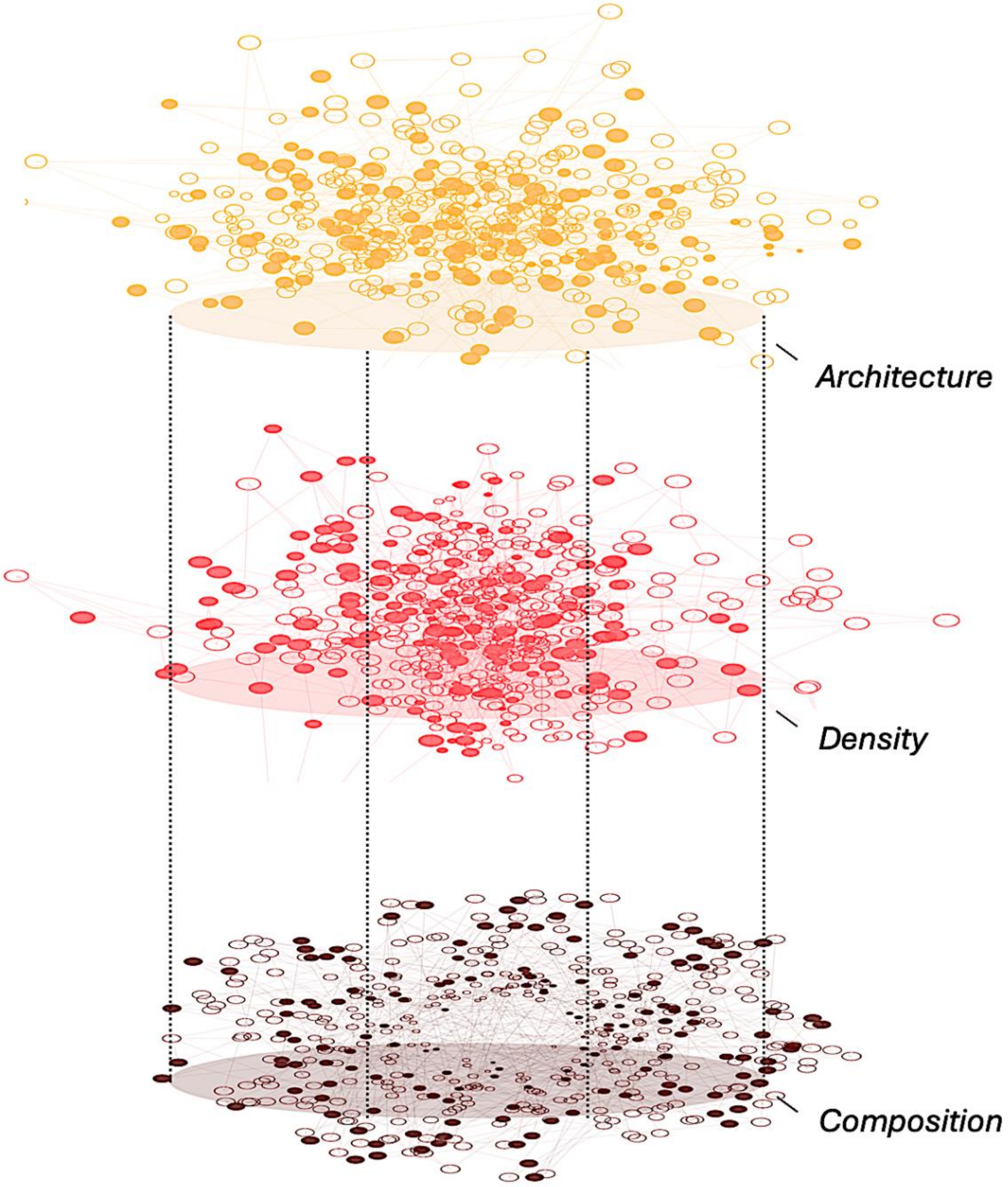


Figure 3. Example of network architecture of galls on a single plant, reflecting relationships between quantitative network layers. Open nodes represent wasp galls, filled nodes represent midge galls. Network graphs for plant architecture (yellow), gall density (red), and gall composition (brown) domains exhibit distinct connectivity, with node size representing gall diameter and clustering between nodes representing stronger interactions between galls.

Conversely, small galls formed on lower nutrient tissues, such as those of *A. acroptilonica*, likely represent weaker sinks. Insects that act as weak metabolic sinks may nonetheless alter plant conditions through alternative mechanisms, such as plant defense activation. Low larval densities like those seen in the wasp are often associated with strategies that minimize intraspecific competition and reduce exposure to plant defense compounds (Borges 2018). Additionally, oviposition can influence plant responses, with endophytic egg-laying (inside plant tissues) potentially triggering stronger defensive reactions compared to surface oviposition due to greater tissue damage (Soler *et al*. 2005).

These life history characteristics illustrate only a handful of the many factors that can influence plant-mediated interactions between insects, with additional factors such as spatial positioning, plant architecture, and population density potentially playing important roles as well (Zhang, Zhong, & Liu 2008).

To capture this complexity, we employed multilayer spatial networks to examine plant-mediated interspecific and intraspecific interactions between the gall midge *Jaapiella ivannikovi* and gall wasp *Aulacidea acroptilonica*. While we did not explicitly measure the mechanisms driving facilitation and competition, such as resource assimilation or defense activation, we developed mathematical models to represent potential plant-mediated effects, approximating the possible influence of resource allocation and plant defense signaling inferred from gall characteristics and spatial relationships.

(*H1*) *Species identity determines the direction and magnitude of plant-mediated interactions.* Based on each species' life history traits, we hypothesized that midges would facilitate wasps but compete with other midges, while wasps would exert competitive effects on both conspecifics and heterospecifics.

(*H2*) *Network domain structure reflects species-specific ecological strategies.* We predicted that galls would exhibit distinct functional roles based on species identity. Specifically, we expected midges to associate more strongly with composition networks, reflecting their dominant influence on gall community dynamics, while wasps would associate more with density networks, reflecting their sensitivity to crowding and density-dependent effects.

(H3) Herbivore species exhibit distinct patterns of network participation across functional domains. We hypothesized midges and wasps would differ significantly in their participation across composition, architecture, and density domains, with midges exhibiting generalist tendencies represented by greater participation across domains. Conversely, we expected wasps to inhabit a more limited functional niche due to constraints imposed by their clustering behavior and anticipated propensity for negative interactions.

(*H4*) *Multilayer network models better predict herbivore performance than single-layer models.* We hypothesized that models incorporating multiple network domains would explain significantly more variation in gall diameter than single-domain models, reflecting the complex, interconnected nature of plant-mediated interactions.

Methods

Experimental Design

We established a common garden of *R. repens* plants at Colorado State University's Agricultural Research Development and Education Center near Fort Collins, Colorado. Plants were started from field-collected rhizomes representing four knapweed populations originating from the western slope, central plains, and northern regions of Colorado. Rhizomes were cut into 10cm lengths such that they included at least one stem-producing node. Twenty-four rhizomes were planted in 6x6 in holes filled with garden soil in May 2021 in a 5 x 20 array. We used a spacing of 2.5 m between plants in all directions to reduce the potential for competition between plants via root exudates or volatile organic compounds (VOCs). Plants were watered for two consecutive hours every other day at a rate of ½ gallon per hour via dripline irrigation. They were then allowed to grow for two months before they were individually placed in fine mesh cages (0.5x0.5x1 m). Insects were released into cages according to one of three herbivore treatment levels: 10 mated female *J. ivannikovi*, 10 mated female *A. acroptilonica*, and an interaction treatment with both insects (5 mated females of each insect).

Data were collected for 23 individuals (excluding one of the original 24 plants that did not survive) at the end of the growing season (mid-October) when insects entered diapause and knapweed senesced. We recorded for each plant: gall density and species identity, spatial arrangement and connectivity of galls, and vascular architecture (branch orientation, lengths). For each gall, we measured diameter (a proxy for insect number), stem identity (main or lateral), height from the plant base, position on the stem, and the shortest paths in cm between all directly connected directly in the vascular network.

To establish the relationship between gall diameter and the number of adult midges that emerge, we recorded insect emergence from midge and wasp galls. All overwintering wasp galls were collected from *R. repens* plants and were maintained at (lab conditions: 25 °C and 16:8 light: dark photoperiod) and larvae were allowed to emerge. Given that midges are multivoltine and emerge from galls throughout the growing season, it was not feasible to collect emerging midges without compromising the experiment. As such, midge galls collected from *R. repens* grown in a greenhouse (27 °C and 16:8 light: dark photoperiod) were measured with calipers to determine diameter. The number of emerged midges was then estimated from the count of larvae, pupae, adults, and egg casings observed in dissected midge galls.

Midge gall diameter was significantly correlated with the number of midges emerging from galls (Figure 5). For wasp galls, relative gall diameter (gall diameter normalized against the globally largest wasp gall) was used to better capture subtle variations in gall size, given that females typically lay only one egg per gall and there is relatively little variation in gall size or insect emergence. Relative gall diameter was significantly correlated ($R^2 = 0.143$; $p < 0.005$) with wasp emergence.

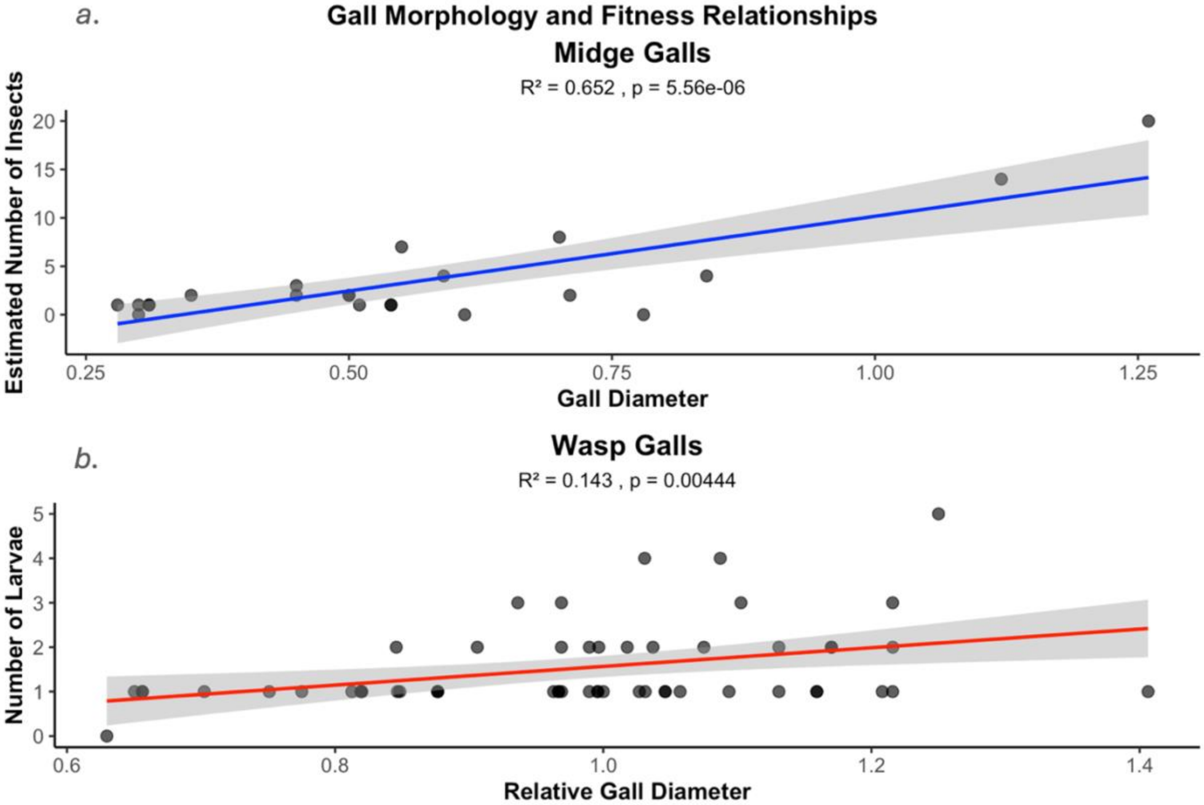


Figure 5. Regression plots for gall morphology-fitness relationships for **(a)** midge (blue) and **(b)** wasp (red) galls. Regression lines indicate correlation.

Spatial Network Generation

All spatial networks were generated using the igraph package (Csardi & Nepusz 2006) in R version 4.2.0 (R Core Team 2024). Each network represents one plant, and within each network *nodes* represent galls connected by *edges* (Zhong *et al.* 2014), the graphical equivalent of stems. Orientation and spatial distances between directly connected galls were used to build network architecture and calculate pairwise distances, producing a set of spatially explicit models spanning three functional domains based on plant architecture, gall density, and gall characteristics (Figure 3).

These *graph layers* as they are known in network analysis, represent distinct mechanisms hypothesized to influence plant-mediated interactions between the gall wasp and midge. Each layer exhibits unique *connectivity*, a binary measure of the presence or absence of an interaction between pairs of galls. For instance, the architecture layer defines connections according to plant vascular pathways, creating a graphical representation of the true branching architecture of sampled plants (Figure 4). Conversely, for the density model, all galls across a plant are connected. However, the *edgeweights* applied to connections determine their direction and magnitude (Zhong *et al.* 2014), such that the characteristics and strength of each gall-gall connection is distinct. For instance, in a spatially defined network, models may assign higher weights to edges between nearby galls than to edges between distant pairs (Zhang, Zhong, & Liu 2008).

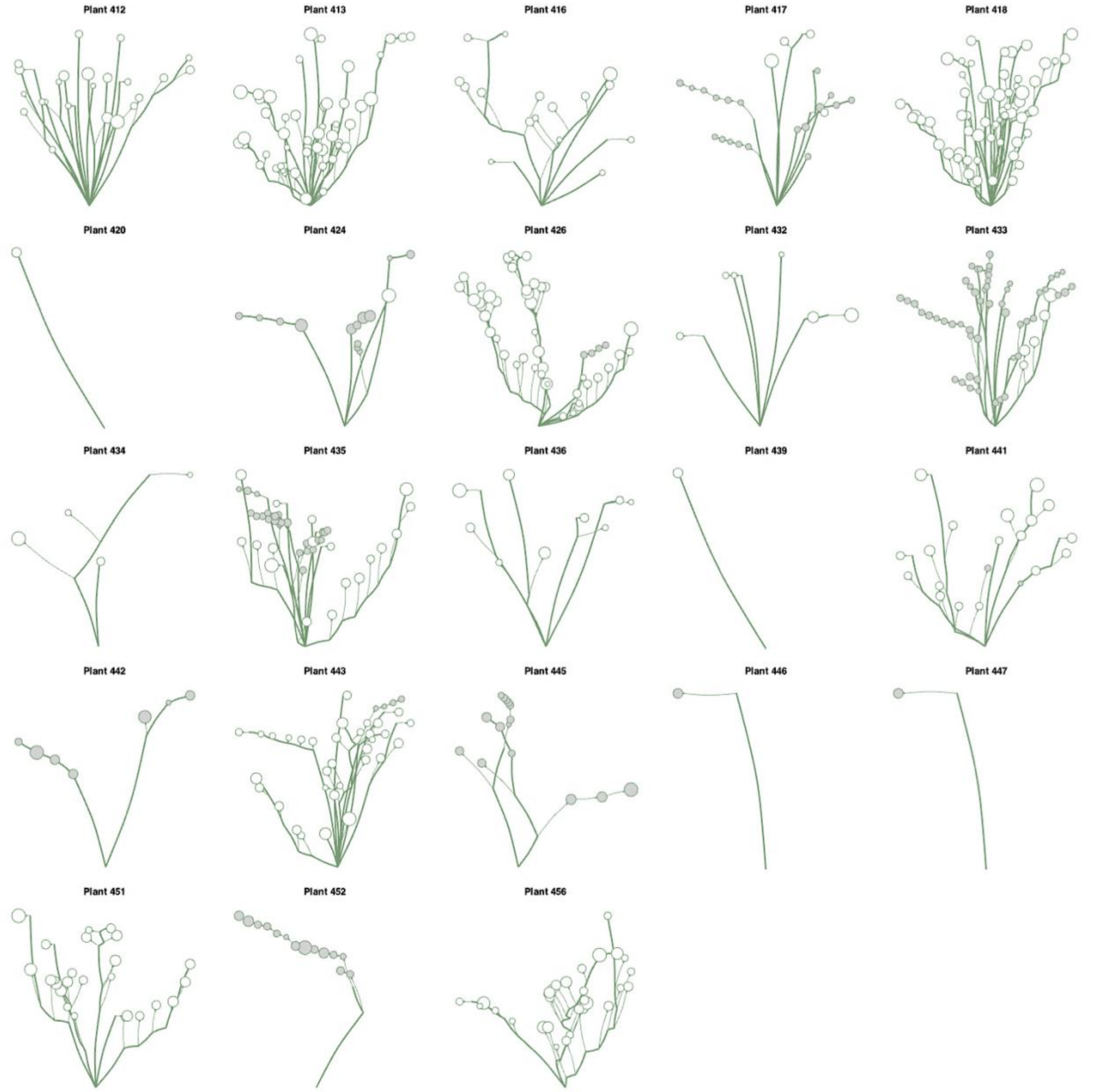


Figure 4. Vascular architecture of gall networks on individual plants. Nodes represent individual galls, with white representing midge galls and gray representing wasp galls. Edges (stems) connect pairs of galls that are considered to interact with each other. These plots depict plant vascular networks based on actual spatial connectivity (i.e., orientation on apical stems or lateral branches, connections to other galls) and measured distances (cm) between all galls across the plant and between each gall and the base of the plant.

To describe plant-mediated interactions between *J. ivannikovi* and *A. acroptilonica,* we constructed a three-layer multiplex network, with each layer representing a distinct interaction domain. We began by developing a set of candidate models representing hypothetical mechanisms that used spatial relationships and gall characteristics to represent:

- **(i)** **Composition:** captures how the composition and species identity of neighboring galls influence focal gall performance;
- **(ii)** **Architecture:** incorporates both the vascular connectivity between galls and the sink strengths of neighbors (gall diameters), representing how shared plant pathways mediate gall interactions;
- **(iii)** **Density:** represents gall density and crowding effects on focal gall performance.

Statistical Analyses

All statistical analyses were performed in R version 4.2.0 (R Core Team 2024). Statistical significance was determined at $\alpha = 0.05$ and error bars represent ±1 standard error unless otherwise noted. To assess gall diameter correlation with insect fitness metrics, we performed linear regression analysis using gall diameter as the predictor and number of emerged midges as the response variable. Because *A. acroptilonica* exhibit little morphological variation in gall size, relative gall diameter (gall diameter divided by the largest wasp gall diameter in the dataset) was calculated for the set of wasp galls and regressed against the number of adults emerging from each gall.

Two-sample t-tests were used to compare participation coefficients and ANOVA were used to test differences in domain proportions between the two gall species. Non-metric Multidimensional Scaling (NMDS) ordination analysis was performed on the network metrics matrix using Euclidean distances with 100 random starts (stress = 0.17), followed by environmental fitting to identify metrics significantly correlated with ordination axes. Domain assignments were determined by quadrant positions in NMDS space and validated with PERMANOVA.

For each domain, participation coefficients (P) were calculated using the formula $P = 1 - \Sigma(k\_i/K)^2$, where k_i represents the weighted degree in domain i and K represents the total weighted degree across all domains, ranging from 0 (all connections in a single domain) to 2/3 (balanced connections across domains), quantifying how evenly a gall distributes its connections across all three domains.

Weighted graphs were constructed with domain-specific connection logic and edgeweights for each candidate model. A full list of models considered for each domain is available in Appendix Table 1. Model parameters were tuned by systematically assessing parameter influence on model performance using signal sweeps (Appendix Table 2). Node weights were calculated as either the sum or average of nodes' edgeweights. Candidate models were evaluated using AICc by regressing node weights against log-transformed gall diameters. Single-layer models were then combined into multilayer models and AICc model selection was used to determine the final global network models.

Two final models were produced to describe local and systemic interactions separately. We screened the initial multilayer ordinary least squares (OLS) fit for influential observations using Cook's distance with a threshold of $\frac{4}{n-k} > 0.008$. Two observations (rows 208 and 358; $D = 0.996$ and 0.113) exceeded the threshold and were excluded from subsequent analyses. The trimmed OLS model still showed significant heteroscedasticity (non-constant-variance (NCV) test: systemic $p = 0.0016$; local $p = 0.0022$). Residuals showed significant deviation from normality (Shapiro-Wilk test: systemic $p = 4.7e^{-6}$; local $p = 4.1e^{-6}$), though diagnostic plots indicated this was primarily due to a few outlying observations in the tails rather than systematic departure from normality. Because residual spread differed by species (midges: $\hat{\sigma} = 0.424$, $n = 347$; wasps: $\hat{\sigma} = 0.334$, $n = 156$) we refitted the models with weighted least-squares (WLS), assigning each observation the inverse-variance weight $w_i = \frac{1}{\hat{\sigma}^2}{}_{species(i)}$. Weighting eliminated heteroscedasticity (NCV test: systemic $p = 0.529$; local $p = 0.464$) without altering fixed-effects structure. The two final models differed only in their penalty terms:

Systemic: $w = S_{max} (1 - e^{-\beta N})\, e^{-d/\lambda}$

Local: $w = -\alpha \quad (d < r) \quad \text{else } 0$

The systemic density penalty model incorporates principles from systemic acquired resistance literature, where each gall primes plantwide defenses that increase sensitivity to subsequent attacks. It combines an exponential decay function to represent signal attenuation with distance (Heil & Ton 2008; Zebelo & Maffei 2015) and a saturation function reflecting dose-dependent defense responses common in plant immunology and defense signaling (Karban & Baldwin 1997; Agrawal 1999). Parameter tuning ensured biologically realistic values, with galls more than 40 cm away having approximately one third the impact of direct neighbors. In contrast, the local defense model applies penalties only between galls within 10 cm of each other representing local competition.

Both models included species identity and its interaction with a three-way interaction term of density penalty, number of heterospecific galls, and number of conspecific galls, differing only in their penalty terms. This resulted in a four-way interaction structure that allowed the relationship between gall density and composition to vary by species at the local and systemic levels.

Results

Herbivores exhibited markedly different patterns across our three interaction layers culminating in network-level differences. NMDS ordination analysis (Figure 5) revealed a well-fitted representation of network structure (stress = 0.17), with significant clustering of galls by domain (PERMANOVA: F = 18.7, $R^2 = 0.32$, $p < 0.001$) and clear separation between gall types (PERMANOVA: F = 9.2, $R^2 = 0.16$, $p < 0.001$), supporting our hypothesis (H2) that network domain structure reflects species-specific ecological strategies.

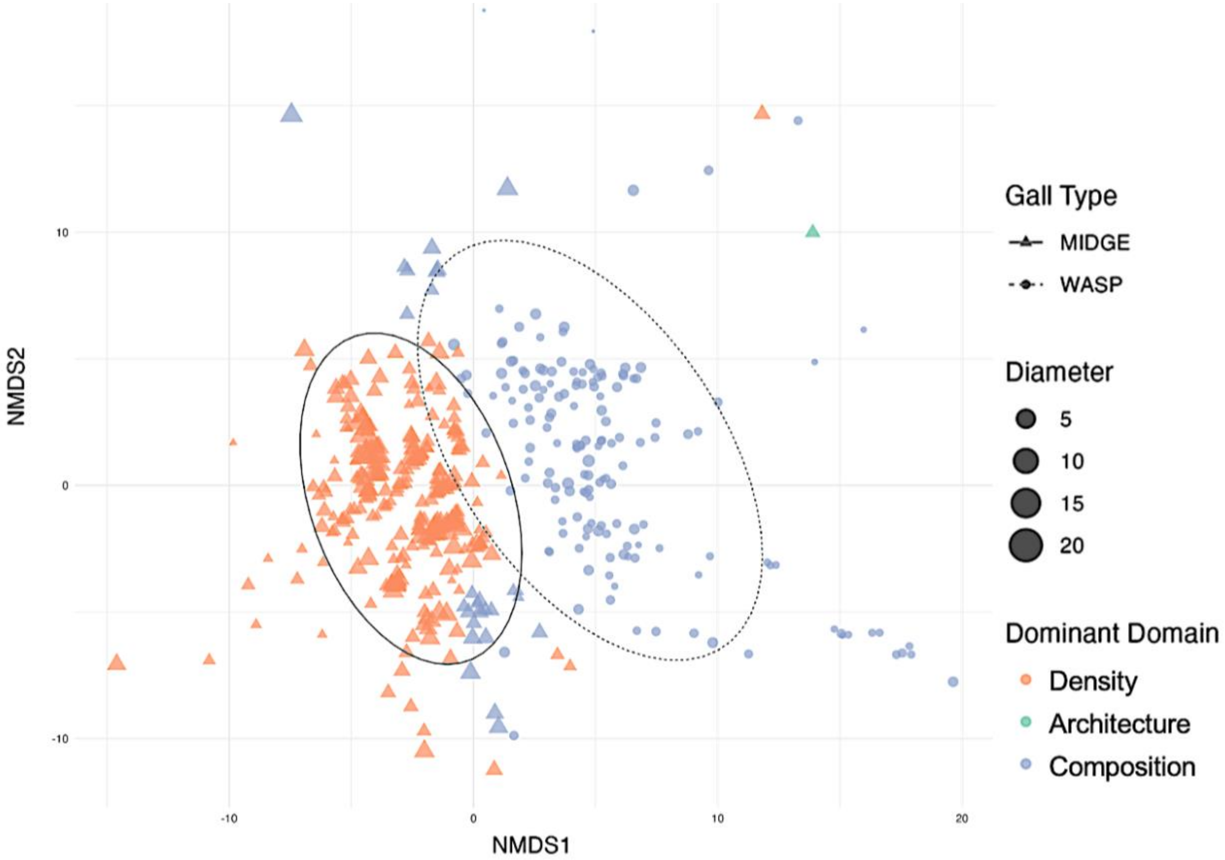


Figure 5. Non-metric multidimensional scaling (NMDS) ordination showing divergence between midge (triangle) and wasp (circle) gall networks across functional domains, with density (orange) and architecture (green) loading primarily on NMDS1, and composition (blue) on NMDS2. Point size represents gall diameter. Ellipses represent 95% CIs.

Overall, species exhibited strong differentiation along NMDS1, reflecting distinct responses to functional domains. The upper left quadrant is defined by positive correlations with density penalties and number of conspecifics across the plant, reflecting that conspecifics buffer density penalties for galls in this quadrant. Midge galls segregate to this quadrant, indicating interspecific facilitation dominates at high gall densities. Conversely, wasp galls clustered to the right, reflecting interspecific competition at high gall densities. Both insects participated weakly in the architecture domain, indicating that for herbivore performance the type and density of neighboring galls are more important than their size or the vascular relationships between galls.

We also supported our network participation hypothesis (H3), observing significant species-specific differences in herbivore integration patterns across network domains (two-sample t-test: $t = 3.86$, $df = 173$, $p < 0.001$; Figure 6). Consistent with our predictions, midges possess a higher mean participation coefficient (0.43 +/- SD 0.11) reflecting that their connections are more evenly distributed across domains whereas wasps are more specialized (0.29 +/- SD 0.10).

Importantly, NMDSI exhibits a strong positive correlation with participation (Figure 7), representing more even participation across domains as NMDSI shifts from negative to positive. This suggests that density penalties are the primary driver of indirect interactions between insect herbivores, and that plant vascular architecture and neighboring gall size become important only at low gall densities.

Cross-validation revealed that combined models consistently explained more variation in gall diameter than domain-specific models, supporting our hypothesis that multilayer network models better explain herbivore performance (H4). Species-specific models explained relatively little variation overall: density and architecture ($R^2 < 0.05$) and composition (*wasp*: $R^2 = 0.02$; *midge*: $R^2 = 0.15$), suggesting differential effects between species.

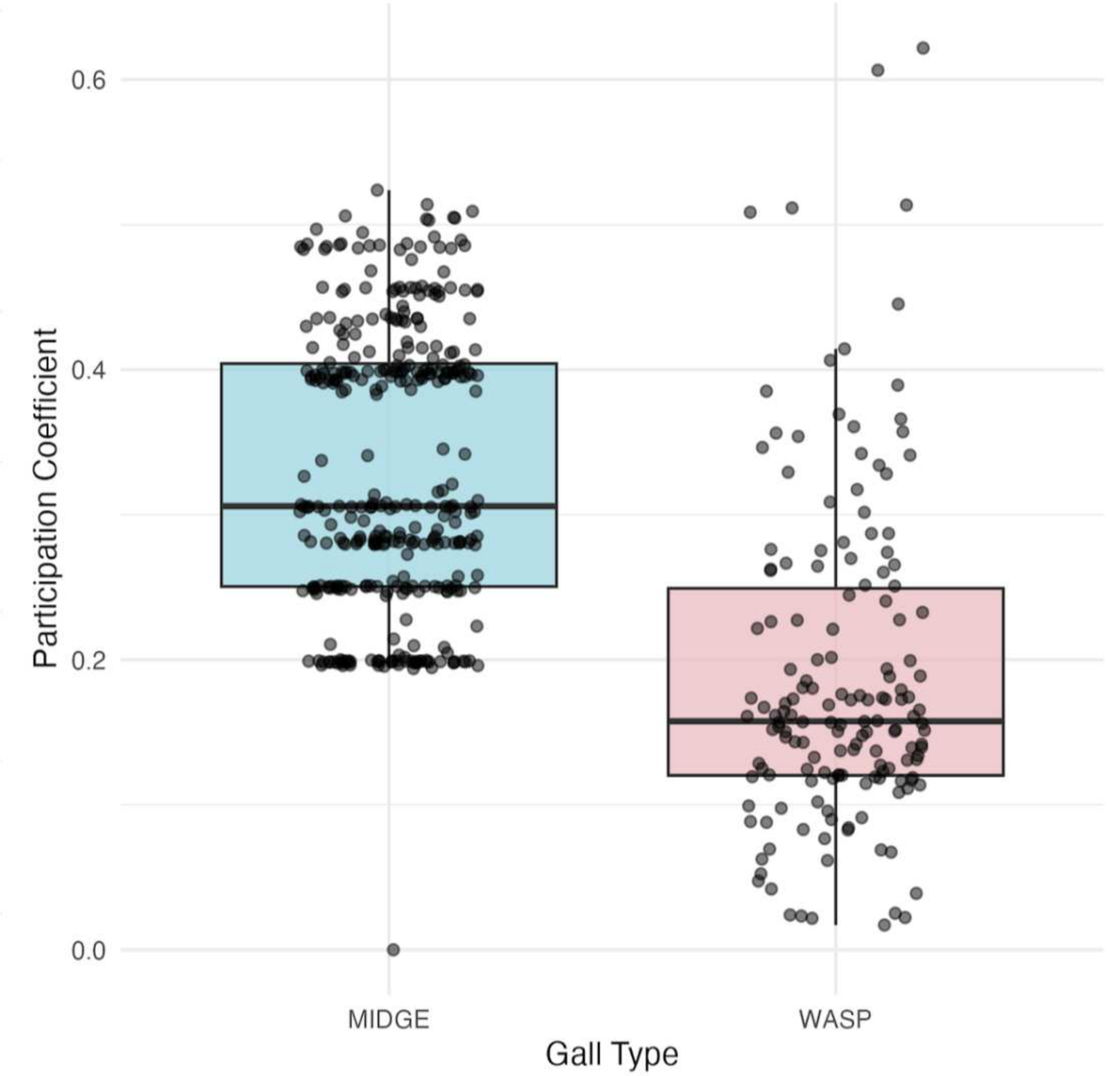


Figure 6. Dot-boxplot of participation coefficient by gall type, where 0 indicates a gall shares connections in only one domain, while 2/3 indicates a gall exhibits equal membership across all domains.

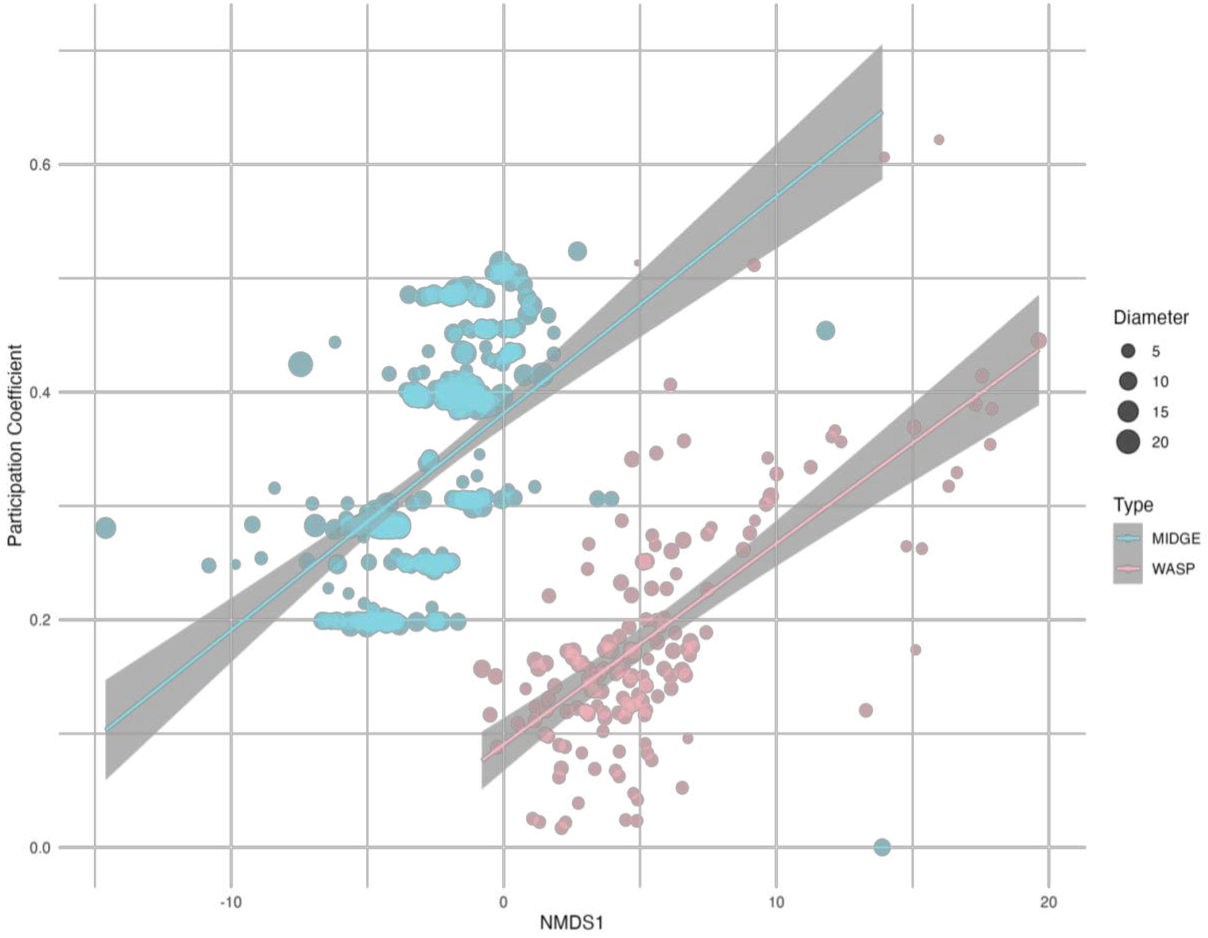


Figure 7. NMDS ordination reveals participation across layers increases as gall density decreases. Point size indicates gall diameter of each midge (blue) and wasp (pink) gall. 95% CI (gray) indicate confidence of each regression across predictions.

Our final models both describe how gall composition mediates density-dependent interactions between species, one at the local level and the other at the systemic level. Among all systemic models considered, our full weighted-least-squares (WLS) model received the strongest support (ΔAICc = 10.4), explaining 94% of Akaike weight. The local-interaction WLS model exhibited similar performance with a ΔAICc 9.8 units lower than the next best model and accounting for 99% of Akaike weight.

The systemic model explained 43.4% of total variation in gall diameter ($R^2 = 0.434$, F = 76.08, $p < 0.001$) while the local model explained 43.2% ($R^2 = 0.432$, F = 75.54, $p < 0.001$). While none of the explicit architecture-only models (Appendix Figure 1) survived model selection, architectural cues nonetheless played an important role in the final models, distinguishing between local and systemic plant-insect interactions.

Both local and systemic models revealed consistent contrasting patterns between species, yielding partial support for our hypotheses about species-specific interaction directions (H1). For the systemic model (Figure 8), wasp galls exerted density-dependent competitive effects on midges ($\beta = -0.016$, $p = 0.002$) and other wasps ($\beta = -0.0027$, $p = 0.002$). Conversely, midge galls not only buffered against density-dependent competition but were also associated with increased gall diameter for both midges ($\beta = +0.0013$, $p = 0.002$) and wasps ($\beta = +0.028$, $p = 0.001$).

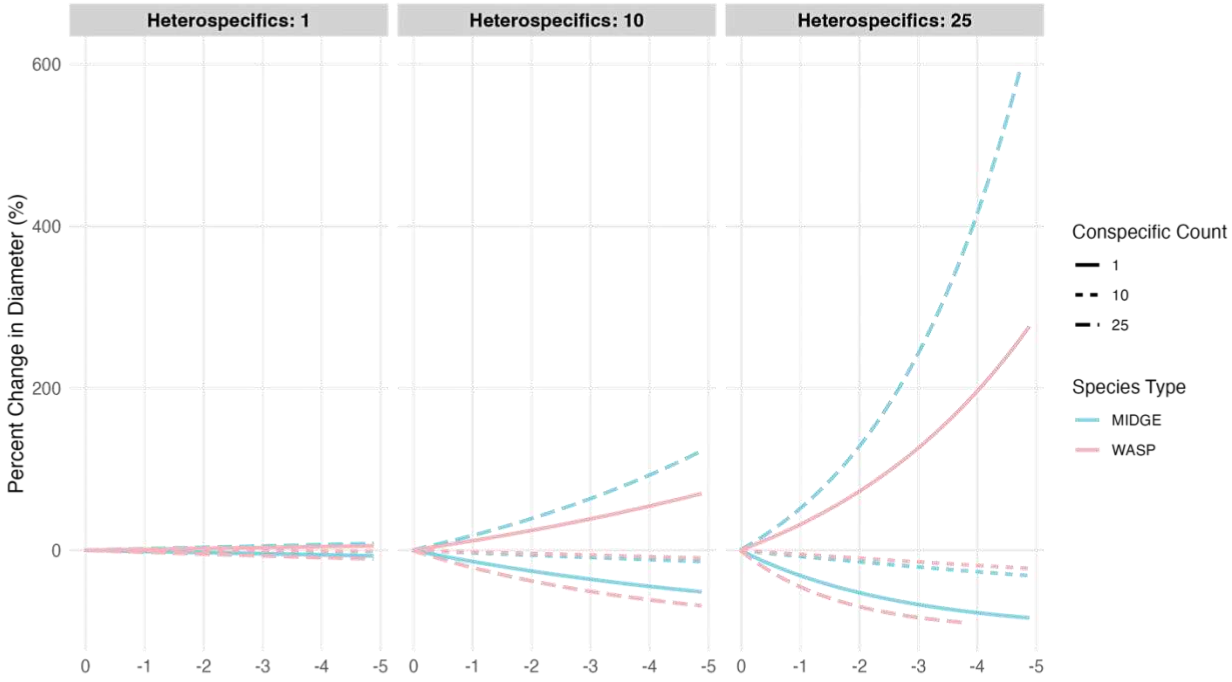


Figure 8. Interaction effects of heterospecific density (by facet plot), conspecific density (solid = 1, dashed = 10, dotted = 25), and systemic penalties on percent change in gall diameter for midges (blue) and wasps (pink).

Similarly, the local model (Figure 9) showed midge galls facilitating conspecifics (β = +0.010, *p* = 0.001) and heterospecifics (β = +0.122, *p* = 0.003), whereas wasp galls exerted competitive effects on conspecifics (β = -0.012, *p* = 0.001) and heterospecifics (β = -0.103, *p* = 0.005). These patterns reveal indirect amensalism at the local and systemic levels, wherein midges facilitate both conspecifics and heterospecifics while wasps exert competitive effects on both conspecifics and heterospecifics.

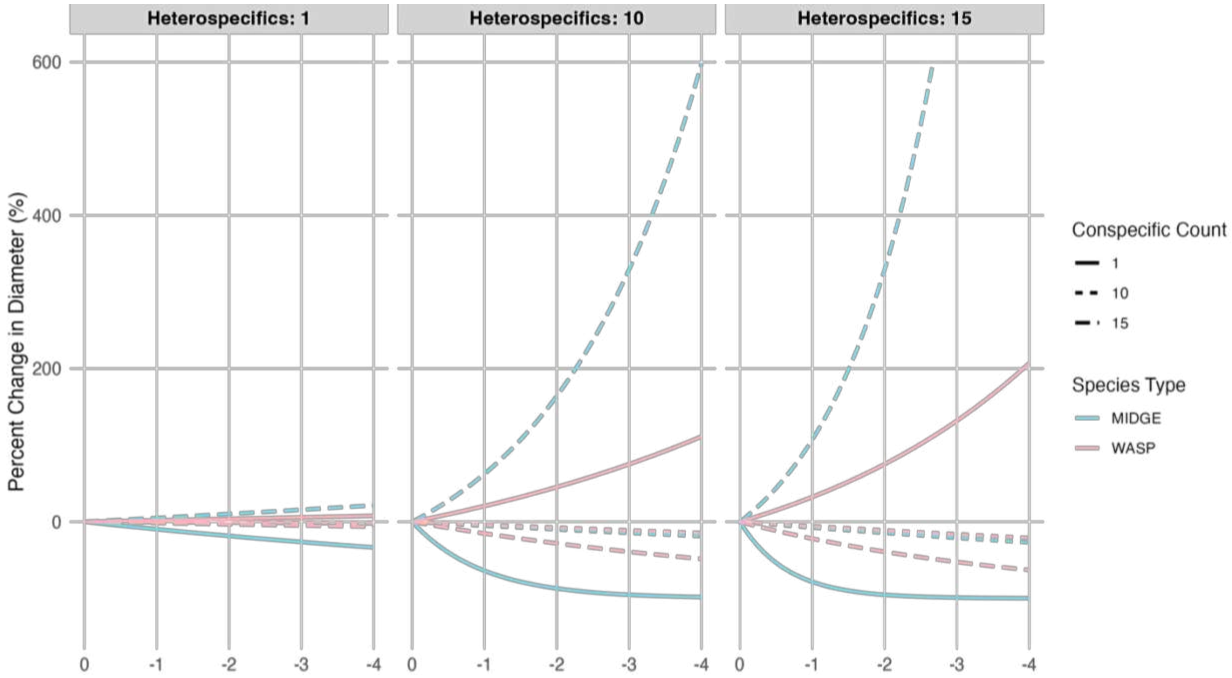


Figure 9. Interaction effects of heterospecific density (by facet plot), conspecific density (solid = 1, dashed = 10, dotted = 15), and local penalty on percent change in gall diameter for midges (blue) and wasps (pink).

These findings are consistent with species-specific patterns of spatial partitioning across the plant (Figure 10). Wasps exhibit close spatial clustering, with a mean nearest neighbor distance of 2.46 steps between pairs of wasp galls, compared to 19.74 steps between pairs of midge galls, and 18.23 steps between heterospecifics. Thus, while wasps may be better equipped to buffer against density-dependent penalties, the frequency and intensity at which they are subjected to local penalties appear to outweigh any competitive advantage they receive from diminishing midge gall fitness. This differential response to gall density and composition aligns with participation coefficient patterns as well as domain utilization differences between gall types.

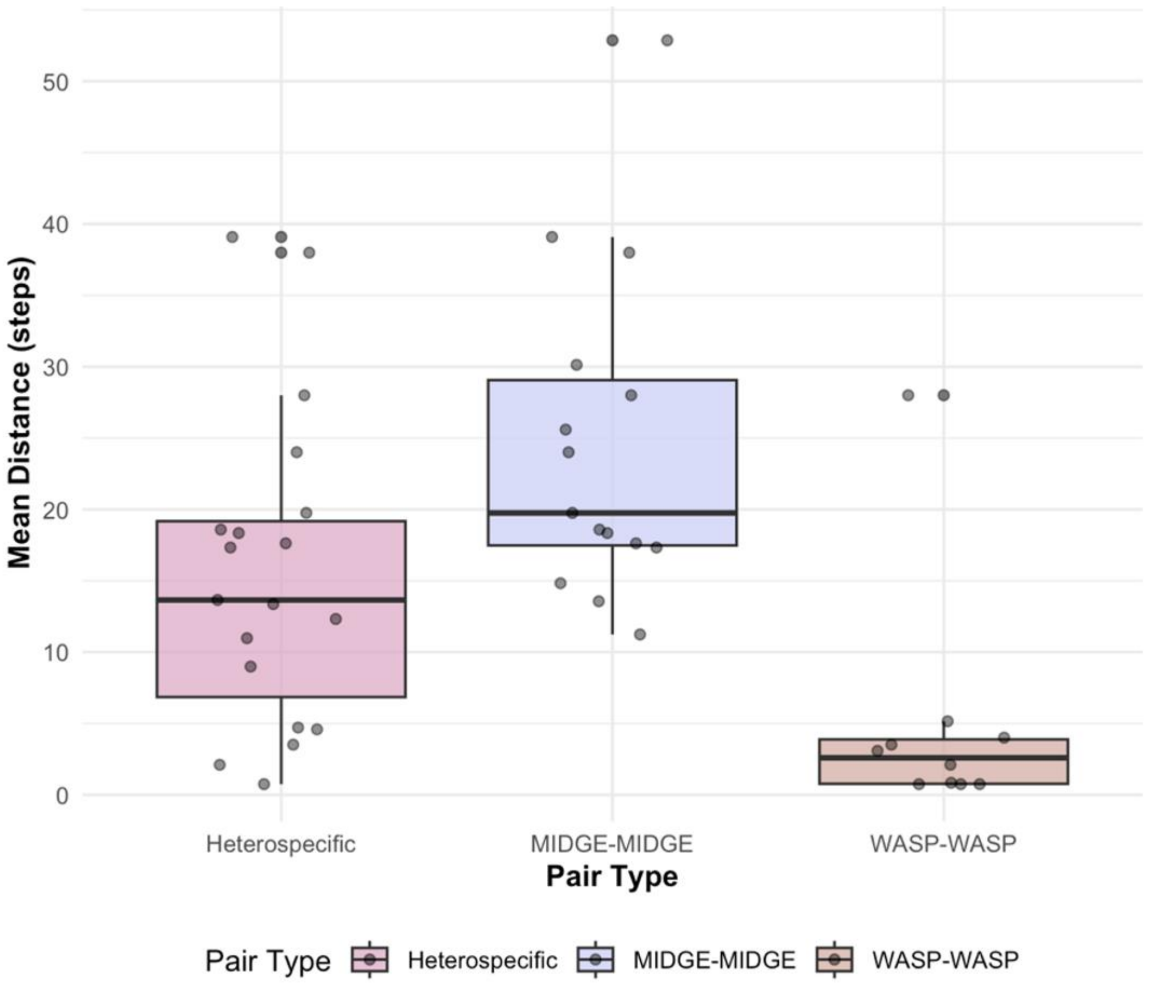


Figure 10. Box plots showing nearest neighbor distance (steps) by interaction type, between the following pairs: wasp-midge (pink), midge-midge (blue), and wasp-wasp (orange). Point data represent mean steps per interaction type per plant. Box plots display median (central line), interquartile range (box), whiskers extending to 1.5x the IQ range, and outliers beyond this as individual points.

Discussion

A 2015 systematic review of the literature on indirect interactions (Sotomayor & Lortie) echoed a sentiment that has persisted since indirect interactions first gained ecologists' attention nearly 50 years ago: the field still lacks effective experimental frameworks for isolating the mechanisms that underlie indirect ecological effects (Holt 1977). This is especially true when examining the effects of indirect interactions on multiple species or trophic levels, where ecological outcomes are often mediated by interactions between physiology, spatial structure, and species-specific traits (Sotomayor & Lortie 2015). Here, we demonstrate that network theory offers a powerful tool for addressing these challenges. By modeling each plant as its own network composed of multiple functional domains, we offer an analytical framework that captures complex, interdependent ecological dynamics with high resolution while providing valuable mechanistic insights.

Using domain-specific quantitative interaction networks, we show that herbivore interactions are shaped by plant-mediated effects associated with vascular architecture and spatial partitioning, gall community composition, and gall density. Combined models consistently outperformed domain-specific models in predicting insect fitness, demonstrating the effectiveness of multilayer network approaches that integrate multiple influences simultaneously. Notably, our models link network structure directly to fitness outcomes through gall diameter, which correlates with insect emergence (Figure 5), providing evidence for the utility of quantitative interaction networks to predict the drivers of species performance in complex communities. This is particularly important for indirect effects, which are notoriously difficult to isolate using traditional statistical approaches (Newman *et al*. 2003).

Our results revealed asymmetric, context-dependent interactions between *Jaapiella ivannikovi* and *Aulacidea acroptilonica* that support a pattern of amensalism. Across both local and systemic scales, midges facilitated neighboring galls regardless of species identity, while wasps consistently exerted competitive effects on both conspecifics and heterospecifics. This asymmetry aligns with species-specific life history traits: midges form large, multi-larval galls that may concentrate resources or suppress plant defenses, benefiting neighbors through shared defenses or enhanced resource availability. Conversely, wasps form smaller, single-larva galls and exhibit tight spatial clustering, potentially intensifying local competition and negative plant responses.

The density-dependent nature of these interactions provides insight into niche partitioning mechanisms. Wasp gall diameter declined sharply with increasing conspecific density, particularly at local scales, consistent with ecological niche theory predictions that organisms with overlapping resource needs compete most intensely when spatially aggregated (Tilman 2004). These findings suggest that indirect interactions are not uniformly distributed across space, but depend on the spatial scale at which mechanisms operate.

Our analysis also revealed significant species-specific differences in network participation patterns, supporting our hypothesis that network domain structure reflects distinct ecological strategies. Midges exhibited higher participation coefficients compared to wasps, indicating more even distribution of connections across functional domains. This generalist tendency may reflect midges' role as strong metabolic sinks that influence multiple aspects of plant physiology, while wasps' more specialized participation pattern aligns with their clustering behavior and sensitivity to local conditions. Furthermore, the strong positive correlation between NMDS1 and participation coefficients suggests that as gall density penalties decrease, galls participate more evenly across domains, implicating negative density-dependent effects as the primary driver of indirect interactions.

The biological control implications of these findings are significant. One of the central questions in biocontrol is whether single-agent or multi-agent strategies are more effective

(Denoth, Frid, & Myers 2002). Our results suggest that multi-agent systems can produce emergent outcomes that are difficult to predict without a mechanistic understanding of plant-mediated interactions. For example, while density penalties reduce wasp gall size, wasps may also benefit from reducing midge competition at the plant level, potentially explaining observed intraspecific competition across stems despite local facilitation under high plant defenses. These dynamics highlight the need for comprehensive assessments of compatibility among biocontrol agents, particularly where indirect effects are concerned.

Our analytical framework is generalizable to other species and systems where indirect interactions are mediated by shared hosts, resources, or spatially structured environments. Qualitative networks can represent any observed interactions, while quantitative networks can incorporate measured interaction metrics like resource flow, feeding intensity, or chemical signaling (Newman *et al*. 2003). Edgeweights can be based on spatial proximity, vascular connectivity, or equations derived from physiological models, making network approaches not only mechanistically insightful but also logistically feasible. While traditional ecological experiments often rely on resource-intensive manipulations or chemical analyses, networks can be constructed with open-source tools like *igraph* (Csardi & Nepusz 2006) using even simple observational data such as a list of interaction partners.

This approach could be particularly valuable for biological control programs, where practitioners must decide whether to release agents separately or together in their introduced range. As such, it should be tested in observational field studies to validate its effectiveness across different systems. Future work should also incorporate empirical measurements of plant resources (e.g., using dye tracers, isotopic labeling, or sap flow measurements) and defense compounds in quantitative networks that assess our proposed mechanisms to establish well-supported quantitative network models that further our understanding of these complex network drivers.

Our findings demonstrate that even small, spatially constrained plant-herbivore communities function as complex adaptive networks where highly local interactions cascade through multiple domains to produce systemwide ecological outcomes. By modeling each plant as its own dynamic network, we provide a new tool for disentangling complex ecological interactions with both theoretical and applied relevance. This approach opens new avenues for studying coevolution, community assembly, and biological control using a framework that is both accessible and conceptually rigorous.

Chapter 3 · Rapid evolution drives increased herbivore resistance in an invasive plant

Introduction

There is increasing evidence that successful invasive species share a key trait: the ability to rapidly adapt to novel environments (Mooney & Cleland 2001; Davis 2005; Facon *et al*. 2006; Koch *et al.* 2014), enabling them to outcompete native species. Rapid adaptive responses can arise through natural selection acting on genetic variation to favor locally advantageous traits and prune disadvantageous traits, or through phenotypic plasticity—the capacity of a single genotype to produce different phenotypes in response to different environmental conditions (Prentis *et al*. 2008; Nicotra *et al.* 2010). Both mechanisms enable invasive species to respond swiftly to novel pressures faced in their introduced range (Maron *et al*. 2004; Davis 2005; Orr 2005). However, invasive species also encounter unique evolutionary challenges. For instance, small populations may experience genetic bottlenecks that reduce genetic diversity, thereby limiting the raw material available for natural selection. Small founding populations often suffer from accumulation of deleterious alleles (Facon *et al*. 2006; Barrett & Schluter 2008). Successful invaders, however, quickly purge these harmful variants (Marchini *et al*. 2016; van der Valk *et al*. 2021), reflecting how rapid evolutionary responses drive adaptive success (Prentis *et al*. 2008).

Life history traits and invasion history are important predictors of invasion success via rapid evolution (Davis 2005). In clonal plants, differentiation is more likely to reflect phenotypic plasticity (Nicotra *et al.* 2010), while sexual reproduction, hybridization, and a history of multiple introductions increase the likelihood that divergence is the result of genetic changes (Bradshaw 1965; Ellstrand & Schierenbeck 2000; Barrett & Schluter 2008; Ward *et al*. 2009).

While numerous studies report genetic changes and restructuring in invasive species and introduced biocontrol agents, the functional outcomes of those changes are poorly understood (Müller-Schärer *et al*. 2020; Phillips *et al*. 2008; Wright & Bennett 2018). Yet, evolutionary changes are likely to occur in invasive or introduced species, especially due to founder effects, hybridization with previously genetically isolated populations or with native species, and evolutionary changes driven by strong selective pressures in response to novel environmental conditions experienced by invasive species (Schierenbeck & Ellstrand 2009).

There is mounting evidence that these scenarios are not only common, but that they have important implications for the ecology and management of invasive species (Phillips *et al*. 2008) as well as the success of biological control programs (Szűcs *et al*. 2012). This is particularly significant given that evolutionary changes can occur over relatively short timeframes, often within a few generations (Koch *et al.* 2014).

Invasive species-biocontrol systems present a unique opportunity to better understand the functional consequences of genetic isolation in host-prey relationships given their unique coevolutionary trajectories (Penczykowski, Laine, & Koskella 2015). Biocontrol agents are screened rigorously for safety to prevent unintended non-target effects after release (Wapshere 1989). Functionally this means that most biocontrol agents are host-specific and therefore have coevolved with their target species (Pearson & Callaway 2005; Brodeur 2012; Esch & Fernandez 2013). At the same time, a lack of alternative hosts in their introduced range means that biocontrol agents must adapt alongside their natural host or evolve to target native species (Futuyma & May 1992). These fascinating relationships also provide an opportunity to examine shifting predator-prey dynamics as climate change is increasingly driving unprecedented range expansions for species with the necessary life history characteristics and genetic diversity for adaptation, and extinction for species lacking these traits (Davis 2005; Barrett & Schluter 2008).

The invasive plant Russian knapweed *Rhaponticum repens* was first introduced to North America in the late 1800s, arriving from Eurasia as a contaminant in alfalfa seed. It has since become widespread, displacing native species and degrading rangeland ecosystems across more than 600,000 hectares of land in the U.S. alone as of 2000 (Marshall *et al*. 2024). Its success may stem from its high adaptive potential, as Russian knapweed possesses several traits predictive of rapid evolution (Bradshaw 1965; Nevo *et al*. 1975; Facon *et al*. 2006; Ruiz *et al*. 2000), including sexual reproduction (Gaskin & Littlefield 2017), multiple introductions, and hybridization between genetically distinct populations in its invasive range (Gaskin *et al*. 2013).

The two Russian knapweed biological control agents, the gall midge *J. ivannikovi* and wasp *A. acroptilonica*, have been approved for release in the U.S. since 2009 and 2008, respectively (USDA APHIS 2008, 2009). After more than a century of separation, descendant populations of knapweed are undoubtedly genetically distinct from their source populations due to hybridization and genetic drift alone, regardless of adaptive evolution (Nevo, Dessauer, & Chaung 1975; Facon *et al*. 2006). Thus, there is a high probability of genetic mismatches between biocontrol agents and knapweed in the introduced range (Keane & Crawley 2002). While invasive species are responding to novel selective pressures in their introduced range, biocontrol agents in the native range continue evolving in response to distinct selective pressures. As long as both parties follow separate evolutionary trajectories, such genetic mismatches become increasingly likely to occur once species are reunited.

Post-introduction evolution of biocontrol agents may therefore be critical for correcting such mismatches. For instance, within only seven years of its introduction to North America, the leaf beetle *Diorhabda carinulata* (Desbrochers), a biocontrol agent of *Tamarix* spp., evolved to shift the timing of diapause induction, enabling better synchronization with host plant phenology and enhancing biocontrol efficacy (Bean, Dalin, & Dudley 2012). Nevertheless, such examples are rare, as evolution in contemporary biocontrol programs remains understudied (Wright & Bennett 2018).

Continuous mass-rearing and redistribution over the past 16-17 years has almost certainly resulted in genetic divergence of both *J. ivannikovi* and *A. acroptilonica* from their Eurasian source populations (Davis 2005; Koch *et al.* 2014). However, the rate of divergence is constrained by each species' unique life history attributes. The multivoltine midge, with 6-8 generations per year in Colorado, has greater adaptive potential than the univoltine wasp. Although R. repens produces seeds each year, its preference for clonal spread and reliance on genetically distinct populations for viable sexual recruitment may constrain its evolutionary potential relative to the obligately sexual wasp, despite possessing similar annual reproductive cycles. Still, its century-long residence in North America, repeated introductions from divergent source populations, and documented hybridization (Gaskin *et al.* 2013) may offset generational constraints. This combination of prolonged evolutionary exposure and increased diversity may have enabled knapweed populations to develop traits that confer increased tolerance or resistance to herbivory.

In turn, this could drive variation in biocontrol efficacy across knapweed populations. Understanding the extent of evolutionary divergence in *R. repens* populations is therefore a critical first step for predicting biocontrol outcomes. It also provides a foundation for examining how host plant evolution may create novel selective pressures on herbivores. To explore these questions, we tested the following hypotheses:

(H1) Rhaponticum repens populations will exhibit genetic divergence in chloroplast genomes consistent with multiple maternal introductions. Chloroplast genomes are highly conserved and evolve slowly. As such, genetic relationships between populations should be independent of their contemporary geographic relationships with clades spanning knapweed's invasive range in Colorado rather than clustering by geography. Additionally, geographically proximal populations should sort into different clades if they originate from different source populations.

(H2) Rhaponticum repens will be more susceptible to herbivory when transplanted to a novel environment. Locally adapted knapweed populations transplanted into a novel climate must redirect resources to abiotic stress responses, limiting resources available to defend against herbivory (Desurmont *et al*. 2011; Dangles, Herrera, & Anthelme 2013; Raman 2021). Consequently, we expect insects to form more galls on transplanted plants relative to plants grown in their home gardens.

(H3) Herbivore performance will be independent of garden climate. Because galls constitute a controlled microenvironment that can buffer against external climatic conditions (Miller, Ivey, & Shedd 2009), host genotype rather than climate should determine herbivore performance.

To test our hypotheses, we isolate environmental effects from genetic effects by performing a reciprocal transplant experiment to examine the effect of host genotype on plant-insect interactions. We also *de novo* assemble the first *R. repens* chloroplast genomes, employing a shotgun next generation sequencing (NGS) approach, resulting in the second complete chloroplast genome assembly for the genus *Rhaponticum* (Boqin *et al*. 2022). Plastome assemblies were then used in phylogenetic analysis to investigate the historical relationships between populations.

Methods

Shotgun DNA library preparation

Leaf tissue samples from 10 randomly selected individuals across 10 wild-collected populations of *Rhaponticum repens* (1B12, 2B12, ARS1, BB4, C3, D4, DB2, P12, W1, WB5) were sampled from between May and July of 2022. Field sites were across Colorado (37.35–40.67 N, 104.70–108.78 W) to represent the range of environmental conditions experienced by knapweed populations in the state. Samples were snap-frozen in liquid nitrogen in the field and stored at –80 °C. Genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen) following the manufacturer's protocol. Extracted DNA was sheared to ~400 bp fragments with a Covaris ultrasonicator, end-repaired and A-tailed, and ligated to Illumina adapters using the Quanta Bio SparQ DNA library preparation kit. Libraries underwent SPRI cleanup, PCR amplification, size selection on a BluePippin, and final quantification by qPCR (see SI). Libraries were denatured and loaded at 20 pM (5% PhiX) on an Illumina MiSeq 2000 (v2 reagent kit; 2 × 251 bp reads).

De novo plastome assembly and mapping

Reads were quality-trimmed with Trimmomatic v0.39 9 (default parameters; Bolger *et al.* 2014) for each population. Trimmed reads passed all FastQC v0.11.9 quality control metrics although did produce flags for per sequence GC content, which revealed a slightly narrow empirical distribution relative to FastQC's theoretical normal curve. This profile is typical for whole-genome libraries from leaf tissue, owing to the high copy number of plastid genomes skewing reads toward low complexity, AT-rich chloroplast sequences rather than more heterogeneous nuclear DNA. Quality-trimmed reads were assembled *de novo* with NOVOPlasty v2.7.2, seeded using the *Rhaponticum uniflorum* rbcL gene (GenBank accession MW683229). Mapping produced closed assemblies for all ten populations.

Each contig was imported into Geneious Prime v2025.0.3 (Biomatters Ltd.). An initial consensus was built by mapping the contig to itself ("Map to Reference"), and the consensus sequence for all ten populations were then aligned to produce a global consensus. To align each population, quality-trimmed reads were mapped to the global consensus using the Geneious mapper under "Medium Sensitivity/Fast."

Phylogenetic analysis

All ten consensus plastomes were aligned to a *Rhaponticum uniflorum* plastome outgroup. The IRa copy of the chloroplast inverted repeat was removed prior to analysis to avoid artificially overweighting IR sites in the alignment. Bayesian inference was performed in MrBayes 3.2.6 (Huelsenbeck & Ronquist 2001) under the HKY85+Γ model (4 rate categories), with 1,100,000 generations including 100,000-generation burn-in, four heated chains (temp = 0.1), sampling every 200 generations, seed = 24028, and GammaDir(1,0.1,1,1) priors on branch lengths. Effective Sample Sizes (ESS) values greatly exceeded recommended thresholds (>200), with values ranging from 500 to 5,000 across all parameters and sampled trees, indicating high convergence and strong statistical support for inferred phylogenetic relationships. A majority-rule consensus tree was constructed from post-burn-in trees, with posterior probabilities of 1.0 across all branches.

Genome annotation

Consensus FASTAs were annotated with GeSeq v2.03 retaining the best annotations only: BLAT references for well-annotated chloroplast genomes from related species (*Lactuca sativa* NC_007578.1; *Centaurea diffusa* NC_024286.1; *Helianthus annuus* NC_007977.1), MPI-MP land-plant CDS + rRNA database, and CDS (*R. repens* voucher XJ-171). tRNAs were predicted with ARAGORN v1.2.38 and tRNAscan-SE v2.0.7; IRs and trans-spliced rps12 were annotated, retaining the best hit. Feature detection in Geneious included: promoter motifs (PROSITE), –35 boxes, Shine–Dalgarno sites, SSRs (Phobos), direct/inverted repeats, ORFs (>300 bp; ATG/TTG/CTG), and coverage anomalies (±2 SD).

Reciprocal transplant

In 2022, two garden sites were established in northern Colorado and western Colorado, the Agricultural Research Development and Education Center in Fort Collins (40.6530463, -104.9972938) and the Colorado Department of Agriculture Insectary in Palisade (39.1131775, -108.3504158), respectively. Field-collected *R. repens* plants from Denver (39.837533, -104.7762), Fort Collins (40.673833, -104.982033), Fruita (39.148591700, -108.320918400) and Delta (39.161008000, -108.782318000) were trimmed to a root size of 10 cm and grown in 6x6x8 in pots in greenhouse conditions (16:8 L:D photoperiod; 20-27°C) to monitor for new growth, ensuring viability. Fifty shoots were randomly selected to represent each population, for a total of 200 plants split evenly across the two garden sites

Shoots were planted in 1-ft deep x 6-in wide holes filled with field soil, with 2.5-m between all plants using a balanced randomized design to ensure equal distribution of populations between the well-watered portion of each garden which were watered every other day for two hours at a rate of ½ gph via drip irrigation and the “drought” portion of each garden which received only rainwater. All plants were caged in 1 x 0.5 x 0.5 m mesh enclosures to prevent insect escape. Insect treatments included a control treatment, 10 female wasps, 10 female midges, 5 female wasps and 5 female midges, and 10 female wasps and 10 female midges that were assigned to plants using a balanced randomized design to distribute them evenly between each combination of water level and plant population. Releases were performed early in the growing season once plants had established and had sufficient growth for insects to gall. At the end of the growing season, gall densities of each insect were measured.

Statistical Analysis

All statistical analyses were performed in RStudio with significance level of $a = 0.05$. As is expected for gall count data, assumptions of normality were violated with counts exhibiting right-skewedness and overdispersion due to zero-inflation (Sidumo, Sonono, & Takaidza 2024). First, factorial logistic regression models were fitted to gall data for each species with a GardenLocation x PlantPopulation interaction term to assess whether zeros differed significantly between treatments, which indicates that they should not be removed. For midge galls the model intercept was 0.31 (SE = 0.40), z = 0.78, p = 0.435 indicating the odds of presence did not differ significantly from zero on the log-odds scale. The log-odds of wasp gall presence did not differ significantly from zero either, with a model intercept was -0.47 (SE = 0.40), z = –1.17, p = 0.244. Likewise, none of the main effects or interaction terms achieved significance for either species so zeros were excluded from count data to alleviate overdispersion.

We then used the R package ‘DHARMa’ (Hartig 2024) to compare candidate error families, including Poisson, negative-binomial, and zero-truncated variants. Simulated residuals were generated for each model and tested for overdispersion, lack of uniformity, and handling of zero inflation. Initial fits showed overdispersion secondary to zero truncation, however zero-truncated models consistently showed poor fit. Accordingly, we subtracted 1 from each count ($y - 1$) to reintroduce zeros then fit a negative-binomial family model. This "zero-excluded" negative-binomial with model exhibited the best fit, with 'DHARMa' diagnostics indicating no significant overdispersion, no departure from uniformity, and no zero-inflation for midges ($p = 0.848$; $p = 0.844$; $p = 0.940$) or wasps ($p = 0.560$; $p = 0.833$; $p = 1.000$). To test our hypothesis that insects would form more galls on transplanted individuals, we fit separate negative binomial models for each species with a GardenLocation x PlantPopulation interaction. To assess the effect of environment on herbivory, gall counts were compared between the two garden sites—ARDEC ($n = 76$ plants) and Palisade ($n = 55$ plants)—using a Welch two-sample t-test with unequal variances.

Results

Plastome assembly and characterization

The chloroplast genome of *R. repens* is 152,562 bp in size consisting of two inverted repeats (IRa and IRb) 25,213 bp, a large single-copy (LSC) region of 84,585 bp, and a small single-copy (SSC) region of 18,559 bp. GC content was 37.8%. We identified 51 exonic regions and 27 intronic regions containing 131 genes, 8 ribosomal RNA (rRNA), and 39 transfer RNA (tRNA).

The circular plastome map confirms that *R. repens* exhibits expected angiosperm architecture (Figure 11), with genes depicted along the outer ring. Gene colors emphasize functional clustering, such as the photosystem-II genes (bright green) found in the LSC region.

The IR regions contain the same set genes in inverted order. The inner ring represents coverage depth, showing uniform coverage across the genome, indicating that read depth did not drop across low-complexity A/T-rich stretches and confirming the *de novo* assemblies are indeed complete.

To characterize the historical relationships among *R. repens* populations, we constructed a phylogenetic tree in 'MrBayes' 3.2.6 (Huelsenbeck & Ronquist 2001) using the annotated plastome of *Rhaponticum uniflorum* (NC_060417; Boqin *et al*. 2022) as an outgroup. Two distinct clades are recovered: Clade A links two western-slope populations (1B12 Delta and 2B12 Gateway) with a northern Front-Range population (D4 Denver). Clade B, by contrast, comprises northern Front-Range (ARS1 Fort Collins, DB2 Mead, WB5 Mead, C3 Denver), central Front-Range (W1 Westminster), western (P12 Delta), and southern populations (BB4 Lasauses).

This topology reflects historical divergence between populations that predates current geography, consistent with multiple, independent introductions of genetically isolated source populations into the same region (Figure 12), supporting H1. For instance, although population P12 and 1B12 are both located in Delta, CO, they fall into separate clades, as do the Denver populations (D4 and C3), demonstrating how historically isolated gene pools come into contact and hybridize within the invasive range, which may facilitate increased genetic diversity and adaptive responses that predict successful invasion.

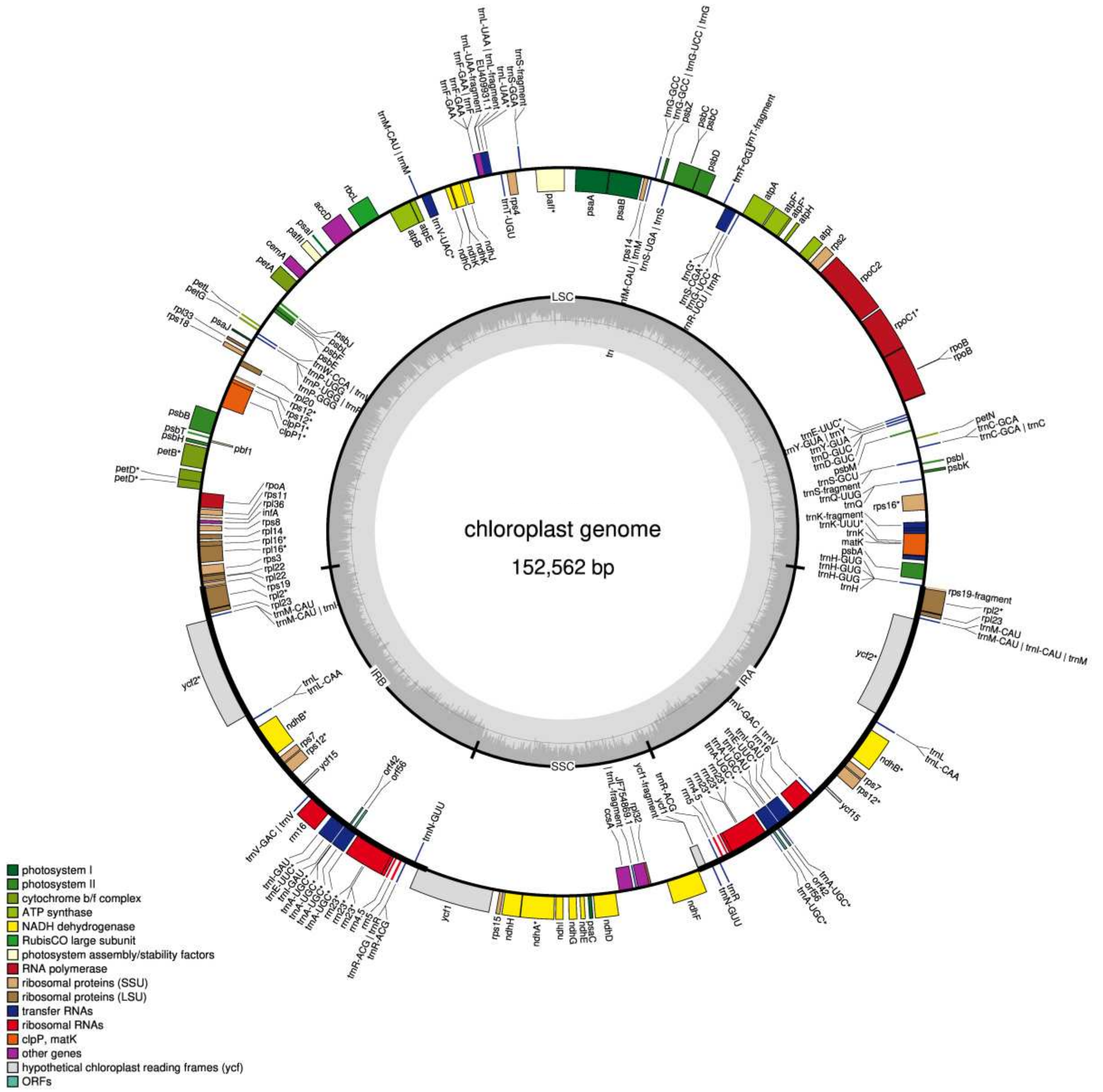


Figure 11. Annotated consensus assembly of *Rhaponticum repens* chloroplast genome produced by OGDRAW v.1.3.1 in GeSeq organelle annotation web tool.

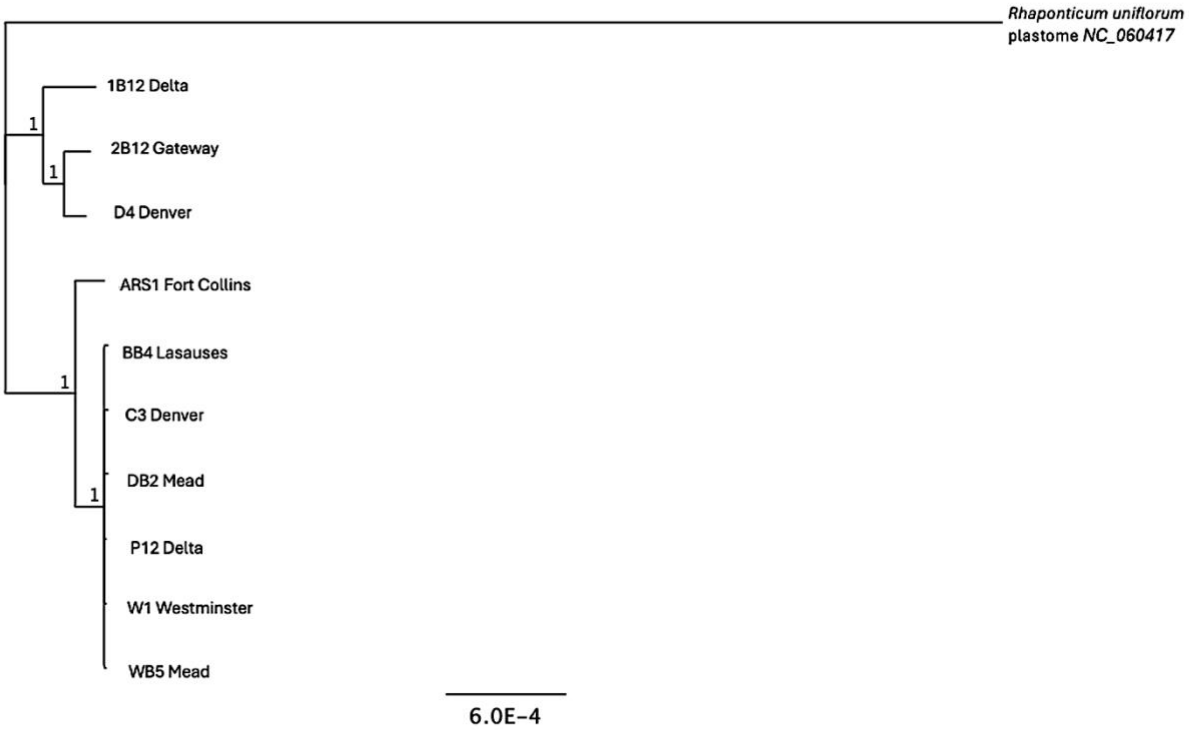


Figure 12. Molecular phylogenetic tree representing historical genetic divergence among knapweed populations and *R. uniflorum* outgroup, branch lengths represent genetic distance, with scale bar depicting $6e10^{-4}$ nucleotide substitutions per site. Posterior probabilities are 1 at all nodes.

Reciprocal transplant

Reciprocal transplant experiments contextualize our findings of climate-driven adaptation in *R. repens* plastomes by revealing strong phenotypic effects on plant-insect dynamics manifesting as greatly increased susceptibility to herbivory, with an average of approximately 17.6 (± 3.9 SE, $t_{(100)} = -4.5$, $p < 0.001$) more galls formed on transplants, strongly supporting H2. Examining the data by regional origin (Figure 13) clarifies the magnitude of transplant effects. Northern Colorado genotypes averaged 26 galls when away, but only 10 galls when at home, with western Colorado genotypes showing a similar pattern (24 away vs 8 home). In both cases, plants transplanted across the Continental Divide in either direction experience a sharp, parallel drop in herbivore resistance. This symmetry indicates that the loss of defense is a property of translocation rather than of any region or historical lineage, suggesting that recent environmental adaptations underlie populations' superior performance in home environments.

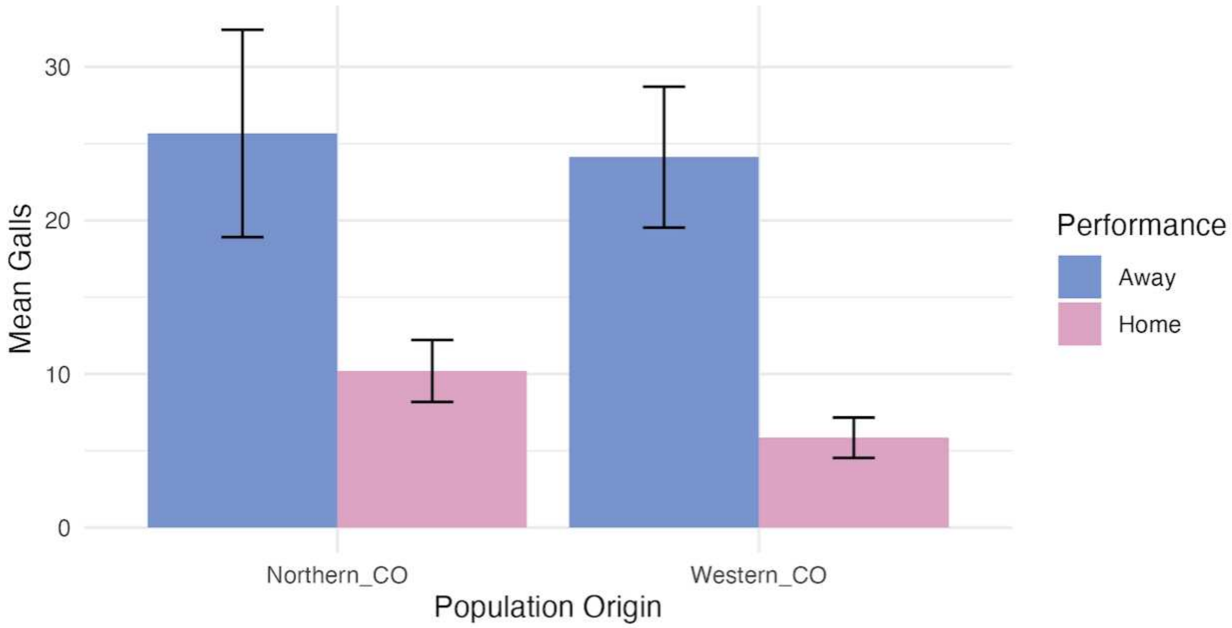
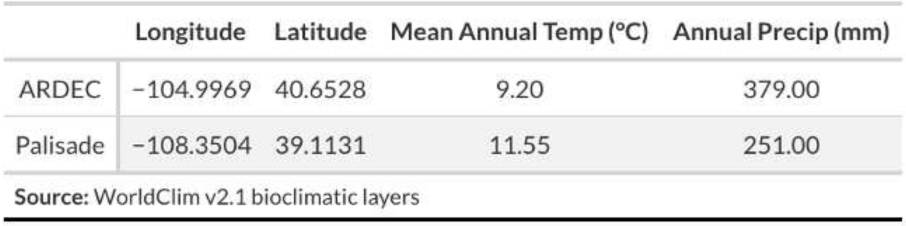


Figure 13. Mean (± SE) number of galls produced per plant for populations from Northern Colorado (left panel) and Western Colorado (right panel). Color denotes garden site, with away sites representing plants grown outside of their region of origin and home sites grown in their region of origin.

Indeed, mean gall load does not differ significantly between the two gardens (ARDEC = 25.9 ± 38.3 SD, n = 76; Palisade = 20.8 ± 34.1 SD, n = 55; $t_{(123)}$ = 0.80, p = 0.424, two-tailed), supporting H3 and indicating genetic rather than environmental influences on performance. Still, the gardens exhibit markedly different climates, with ARDEC being cooler (9.2 °C) and 50% wetter (379 mm) than Palisade (11.55 °C; 251 mm).

Table 1. Annual mean temperature and precipitation for both garden sites.



	Longitude	Latitude	Mean Annual Temp (°C)	Annual Precip (mm)
ARDEC	−104.9969	40.6528	9.20	379.00
Palisade	−108.3504	39.1131	11.55	251.00

Source: WorldClim v2.1 bioclimatic layers

Examining the data by herbivore species and knapweed source location yields additional insights (Figure 14). Firstly, we see that the home versus away pattern holds not only across genotypes, but also across herbivores, with both insects forming a greater number of galls on away populations at both the northern ARDEC garden and western Palisade garden.

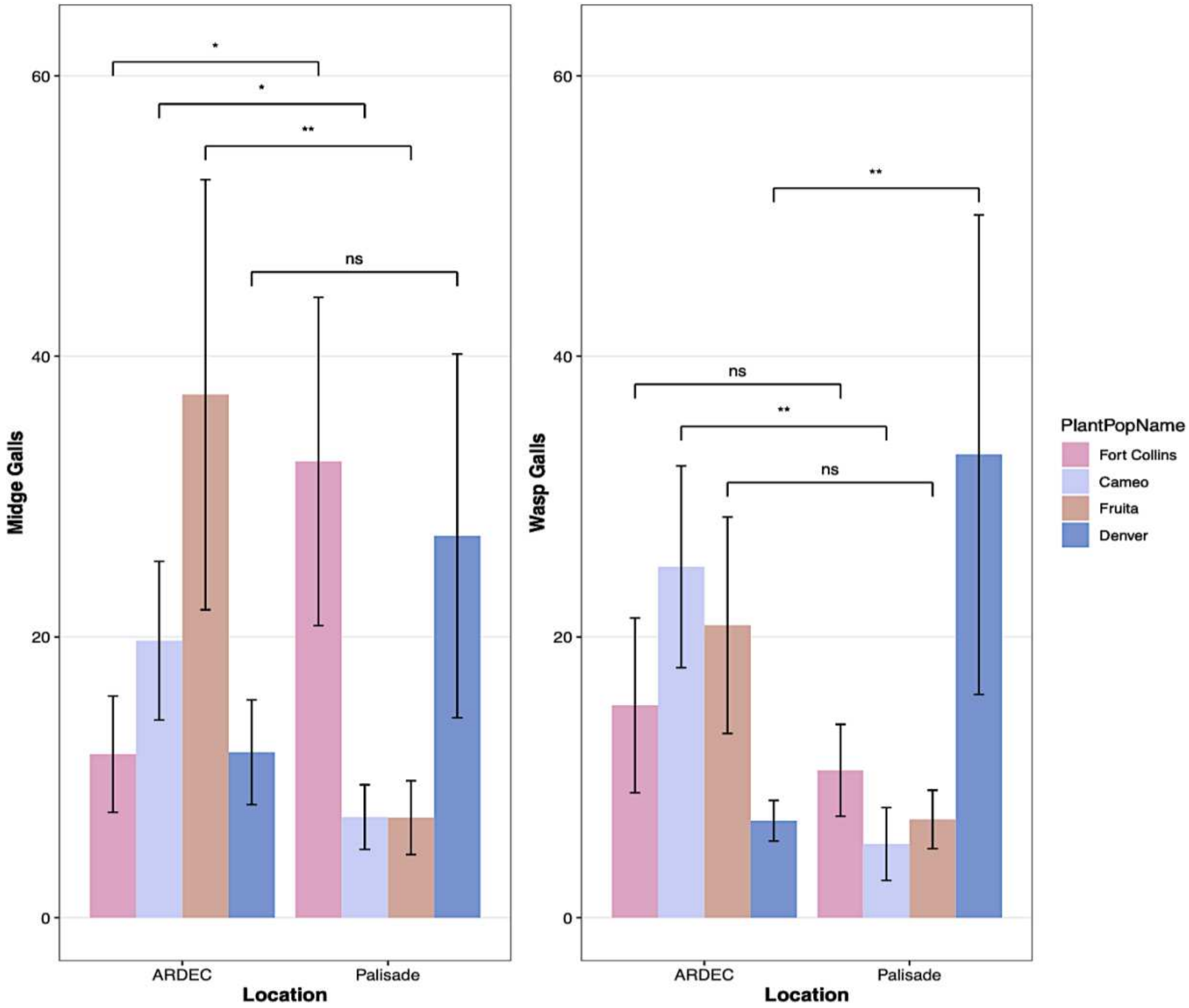


Figure 14. Reciprocal transplant results demonstrating that *R. repens* exhibits increased susceptibility to herbivory (mean gall formation ± SE) by both midges (left panel) and wasps (right panel) when located at an away garden compared to their home gardens. Plant populations from Fort Collins (pink), Cameo (light blue), Fruita (orange), and Denver (dark blue) were reciprocally transplanted among locations. Statistical comparisons between locations are indicated by horizontal brackets above bars. Significance levels: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, *ns* = not significant ($p \geq 0.05$).

Discussion

Although phylogenetic analyses reveal that historic evolutionary divergence of *Rhaponticum repens* populations is decoupled from contemporary geography, reciprocal transplant experiments exhibit distinct ecotypes associated with unique evolutionary strategies. These findings support our hypothesis that invasive populations have undergone rapid evolutionary changes since arriving in North America nearly 120 years ago, and that their evolution shapes plant fitness by altering plant-herbivore interactions.

These findings are particularly relevant in the context of invasion biology and climate change. Invasive success is predicated on the ability to tolerate unfavorable environmental conditions, often in highly disturbed areas with low biodiversity, favoring stress-tolerance and generalist evolutionary strategies (Davis 2005; Facon *et al*. 2006). In such settings, genetic diversity is key to survival (Muhlfeld *et al*. 2014), providing the raw material for selection to act upon and thus maximizing the likelihood an organism will be able to tolerate unpredictable conditions. Our data imply *R. repens* maintains or generates sufficient genetic diversity to for local selection to act on.

This has important implications for knapweed management, with selection for increased genetic diversity predicting successful range expansions into novel environments and tolerance across a broad range of environmental stressors (Szűcs *et al*. 2012). Moreover, knapweed's ability to adapt to novel conditions may foreshadow 'release' from its biological control insects if they do not exhibit similar stress tolerance and evolutionary plasticity (Keane & Crawley 2002). Conversely, understanding the drivers of knapweed evolution and their fitness effects may pave the way to developing climate-optimized biocontrol strategies that capitalize on stress-induced vulnerability to herbivory (Hilbert *et al*. 1981), as demonstrated in our reciprocal transplant experiments.

The observation that plants are significantly more susceptible to herbivores when transplanted suggests that locally adapted or host genotype-matched biocontrol agents may be more effective. However, current bio control programs rely on mass, weird insects that are distributed across broad geographic ranges without regard for local adaptation. Establishing populations that are adapted to local environmental conditions could help maximize efficacy while minimizing the need for repeated releases.

Overall, these results provide support for our hypothesis that *R. repens* exhibits distinct ecotypes with recent evolutionary changes imposing fitness consequences for both knapweed and its herbivores. Our findings also implicate *R. repens* as a candidate for additional work investigating evolution in invasive species, eco-evolutionary dynamics between coevolved species, and the impact of climate change on these dynamics. A particularly valuable next step would be to sequence the *R. repens* nuclear genome and link candidate loci with environmental variables to pinpoint the mechanisms that drive adaptation. Publishing the first complete chloroplast genome for *R. repens* also facilitates further research involving the plastome. Plastome-based approaches lower the barrier of entry for genomic analysis of non-model organisms, considering the cost-savings of sequencing chloroplast genomes relative to plant nuclear genomes, which are orders of magnitude larger, often exceeding 100 Gb compared to ~150 kb for chloroplasts.

Finally, insects themselves are capable of rapid evolutionary shifts over just a few generations (Bean *et al.* 2012; Wada-Katsumata, Silverman, & Schal 2013; Koch *et al.* 2014; Machado *et al*. 2021; Schoville *et al*. 2018). Given the tightly linked coevolutionary histories of

R. repens, J. ivannikovi, and *A. acroptilonica*, this system presents an opportunity for future research investigating plant-insect coevolution combining -*omics* and experimental approaches. Future work should sequence the genomes of midges and wasps collected from field sites where insects arrived via controlled introductions with known establishment timing to examine how ecotype selection influences plant-herbivore dynamics along temporal and environmental gradients. Additional genotype-phenotype association studies should manipulate environmental variables in the greenhouse setting to identify climate-associated genetic variants that influence fundamental plant fitness measures and plant-insect interactions. As the rate of environmental change rapidly accelerates and species invasions increase globally (Carroll *et al*. 2007; Maron *et al*. 2004), understanding rapid evolutionary dynamics is becoming increasingly important for predicting invasion success, identifying areas with the greatest vulnerability to invasion, predicting ecosystem responses to invasions, and developing effective management strategies.

Chapter 4 · Climate gradients shift plant-herbivore network dynamics

Introduction

Environmental stressors can shape ecosystems with profound effects across spatial and temporal scales, influencing everything from individual behaviors to community dynamics and evolutionary trajectories. In addition to efforts to understand the scope, rate, and severity of ecosystem responses to anthropogenic climate change, our ability to accurately forecast how populations will respond is critical to the future of biological conservation, agriculture, and public health (González-Tokman *et al*. 2020; Harvey *et al.* 2020; U.S. Environmental Protection Agency n.d.). Central to this challenge is determining which species are likely to persist, shift their geographic ranges, or face extinction. However, complexity increases with biological realism, particularly when considering the influence of biotic interactions, evolutionary histories, and the potential for phenotypic plasticity or rapid adaptation. Still, because organisms shape and are shaped by their ecological communities it is essential to use frameworks that account for multitrophic feedback loops when studying the effects of global change (Facon *et al*. 2006).

Ecological theory offers insight into how species interactions may change across climatic gradients. One widely studied framework, the Stress Gradient Hypothesis (SGH), posits that facilitative interactions among organisms become more common under harsh environmental conditions (Bertness & Callaway 1994; Brooker *et al*. 2008). Conversely, competition is theorized to be more frequent under relaxed conditions since resource-sharing is less important for survival. However, recent studies challenge this model, suggesting that facilitative interactions can collapse under extreme environmental stress, especially when organisms reach physiological thresholds that limit cooperation (Michalet *et al*. 2006). For instance, under conditions of intense drought, even typically facilitative plant-plant or plant-insect relationships may break down, shifting the balance toward competition or collapse. As primary producers, plant responses to climate change are key to predicting how communities will shift, as changes in interactions with primary consumers often drive cascading effects through trophic networks (Dyer & Letourneau 2013).

Among the factors that influence vulnerability to environmental stressors is community connectivity. Communities with sparse connections face greater risk of collapse, since the loss of even a few species can fragment entire networks. Indeed, redundancy in ecological networks is among the strongest predictors of resilience to stressors, including, but not limited to, environmental changes (Biggs *et al*. 2020). Thus, specialist species can serve as indicators of network vulnerability (Clavel, Julliard, & Devictor 2011). Beyond network structure, the intrinsic traits of species also determine their vulnerability to environmental stress.

Generally, *r*-selected species, characterized by rapid generation times, short lifespans, and tolerance of extreme and variable conditions, are more capable of responding to stressful conditions both via phenotypic plasticity and rapid selection (Albaladejo-Robles, Böhm, & Newbold 2023). This is because the unstable environments that *r*-selected species inhabit select for increased stress-tolerance. Meanwhile, phenotypic plasticity is itself an evolved trait and is favored in heterogeneous environments (Bradshaw 1965; Murren *et al*. 2015). Many invasive species are considered to be *r*-selected (Duyck, David, & Quilici 2007; Valéry *et al*. 2009), given their ability to thrive in disturbed environments where native species struggle to survive and are easily outcompeted (Davis 2005; Facon *et al*. 2006). Indeed, climate change is a major driver of biological invasions (Muhlfeld *et al*. 2014; Harvey *et al*. 2020), resulting in compounding negative effects on native species due to both abiotic and biotic stress from invaders.

Here, we examine the interactions between an invasive plant, *Rhaponticum repens* (Russian knapweed)*,* that exhibits rapid climate-induced evolution (see chapter 3) and its two specialist herbivores—the gall midge *Jaapiella ivannikovi* and gall wasp *Aulacidea acroptilonica*. Both species are highly coevolved with *R. repens*, having been introduced from the native range as classical biocontrol agents (Djamankulova *et al*. 2008). Given that the wasp and midge are highly host range-restricted, they must metaphorically "sink or swim," keeping pace with the rapid evolutionary trajectory of *R. repens* to avoid extinction. This highly coevolved multitrophic network therefore provides a useful model for understanding how climate-mediated shifts affect specialist networks with strong, but sparse, ties. We tested the following four hypotheses:

(H1): Environmental stress differentially affects knapweed performance depending on herbivore presence. We predict that knapweed will show reduced abundance under combined abiotic stress and herbivory compared to either factor independently, but that the magnitude of the effect will vary between herbivore treatment (both insects, only the wasp, or only the midge). We use linear models to test this hypothesis, which we explore using thermal and water performance curves across different herbivore treatment combinations.

(H2): Climate-induced impacts on host plant fitness produce species-specific responses that reflect the unique ecological strategies of herbivores. Previous experiments have demonstrated that wasps induce negative plant-mediated effects more strongly than midges and are correspondingly more negatively affected by these effects, whereas midges exhibit facilitative interactions under these highly competitive conditions (Chapter 2). Thus, we propose that wasp performance is primarily defense-limited (benefiting from stressed plants with reduced defenses; Futuyma & May 1992), while midge performance is primarily resource-limited

(benefitting from unstressed, resource-rich plants). We test this via time-lagged dynamic Structural Equation Modeling (SEM) to establish causal links between environmental stress, knapweed abundance, and herbivore performance.

H3: Climate change will drive geographic divergence of wasp-knapweed and midge-knapweed populations.

I. We predict that under mild climate scenarios, current plant-insect community dynamics will persist with moderate range shifts. However, under severe scenarios, we expect increasing geographic separation between herbivore populations as areas where both species are able to persist become less common.

II: We also predict that the distinct life history strategies of each herbivore will result in different climate vulnerabilities. Specifically, wasps are univoltine, emerging early in the growing season (beginning in April) when conditions may be more favorable, and developing larvae are insulated from environmental effects throughout the remainder of the year (Block 1990). In contrast, midges are multivoltine with 4-8 week generation times throughout the growing season (Djamankulova *et al*. 2008), and form exposed rosette galls among plant leaves, thus experiencing greater environmental exposure across all life stages. Thus, under more severe climate scenarios, we expect that midges will exhibit greater range contractions relative to wasps. To assess these hypotheses, we use bioclimate modeling to produce maps of future species distributions across three climate change intensity scenarios.

H4: Environmental stress alters host quality, influencing plant-herbivore interactions. Insect herbivores are affected by many of the same factors that shape plant community interactions. However, while the SGH is broadly supported across plant communities (He, Bertness, & Altieri 2013), its role in shaping interactions among other taxa, including herbivores, is poorly understood (Dangles *et al*. 2013). For instance, abiotic stress can weaken plant defenses, increasing vulnerability to herbivory. However, previous experiments have demonstrated that under favorable environmental conditions, midge-wasp interactions are mediated primarily by plant density-dependent penalties consistent with defense activation (Chapter 2), with facilitative interactions between the wasp and midge increasing with plant defense intensity. Thus, insect herbivores encounter increased abiotic stress and decreased biotic stress simultaneously. Applying the principles of the SGH, increased abiotic stress could be expected to reduce competitive interactions among herbivores. However, this creates conflicting predictions in our system: while reduced plant defenses under stress should decrease biotic stress for herbivores (potentially increasing competition), the simultaneous increase in abiotic stress should promote facilitation. The net effect of these opposing forces on herbivore interactions remains unclear.

Methods

Experimental design

We monitored 27 field sites across a range of climatic conditions in the state of Colorado, including control and experimental sites (Figure 15). Observations were made across an 11-year period, from 2012-2023, capturing environmental variation across spatial and temporal measures. Control sites had knapweed only, experimental sites had knapweed plus 1) the wasp only, 2) the midge only, or 3) both insects. Plots were monitored along a transit, using a square meter quadrat to assess the same plants annually. We measured knapweed percent cover as well as gall abundance at the quadrat-level.

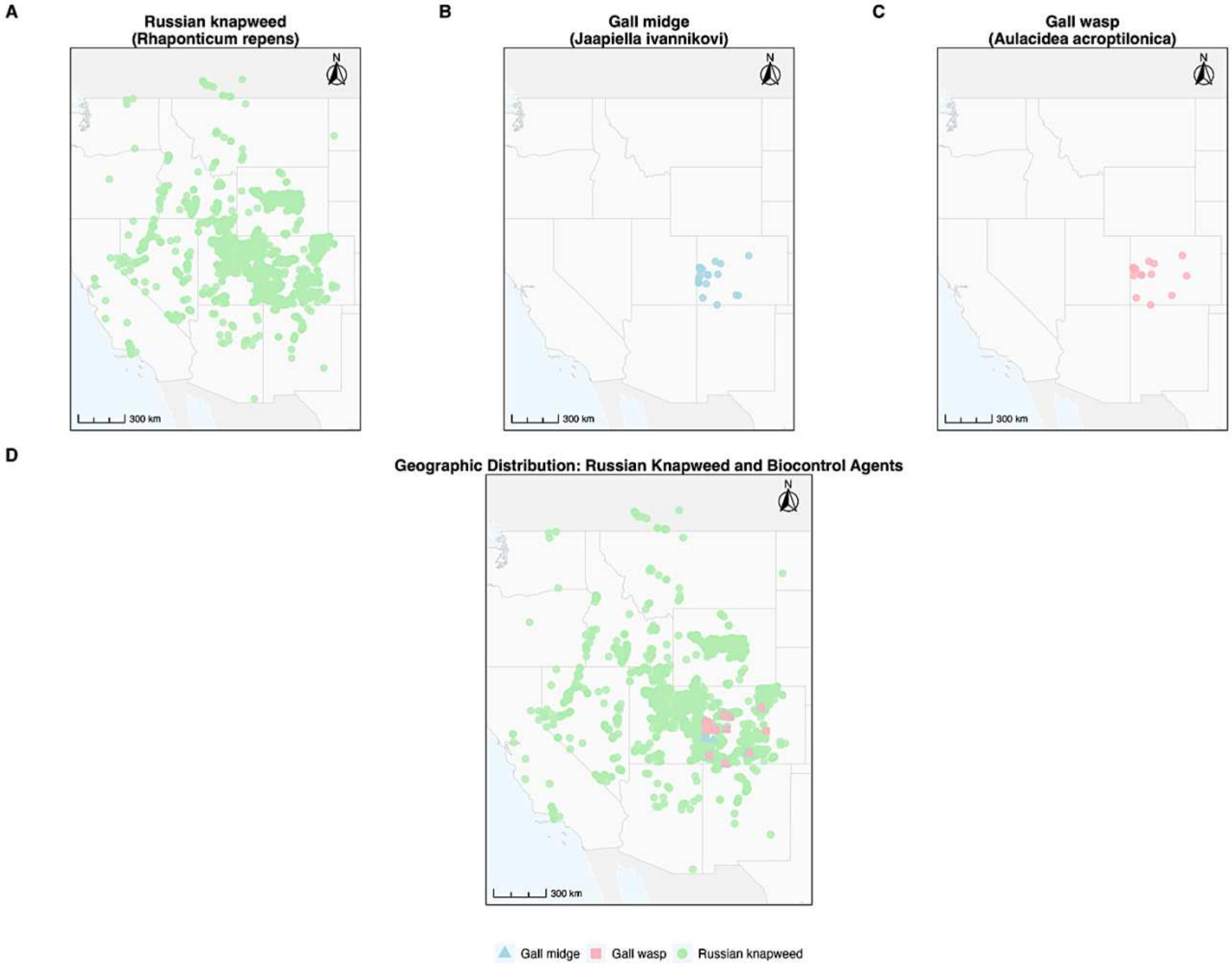


Figure 15. Russian knapweed distribution across North America using ground-truthed Colorado observations and validated EDDMapS records (**a**). Gall midge (**b**) and gall wasp (**c**) release sites across Colorado. All release sites shown on a single map (**d**).

Mapping

We employed ensemble machine learning approaches to predict species presence with high-resolution climate variables from WorldClim v2.1 at 2.5 arc-minute resolution, which were downloaded to R using the 'geodata' package (Hijmans *et al.* 2025). Over 108 predictors were extracted for initial screening, including 19 bioclimatic indices that represent annual trends, seasonality, and environmental extremes, along with monthly data on temperature, precipitation, solar radiation, vapor pressure, wind speed, and elevation. To enhance predictive power, we used feature engineering to produce five additional climate variables including temperature-precipitation interactions, seasonality interactions, temperature stress indices, aridity indices, and thermal stability measures. Feature selection was performed using the 'Boruta algorithm, which identifies variables with importance significantly higher than random permutations. For datasets exhibiting class imbalance (ratio > 3:1), we applied class weighting and scale-positive-weight adjustments to prevent bias toward majority classes. Knapweed presence data was complemented with verified EDDMapS observations of knapweed from 2020-2025 to ensure adequate training to predict pseudoabsences.

Our ensemble modeling approach combined three algorithms: Random Forest (1,000 trees, optimized mtry), XGBoost (300 rounds, $\eta = 0.05$, max depth = 7), and Naive Bayes, with ensemble weights of 45%, 35%, and 20%, respectively. Classification thresholds were optimized using F1-score maximization, testing thresholds from 0.1 to 0.9 in 0.02 increments. Model performance was evaluated using 80/20 train-test splits with stratified sampling. Pseudoabsences were generated for knapweed data, limiting absences to locations at least 50 km from any known knapweed population. Statistical significance was assessed via permutation tests ($n = 500$) comparing observed AUC values to null distributions. We calculated accuracy, balanced accuracy, sensitivity, specificity, precision, recall, F1-score, and area under the ROC curve (AUC) for comprehensive performance assessment.

Across all models, 35 unique variables were selected as important for climate envelope modeling, with the most common including temperature seasonality, isothermality, precipitation extremes, and radiation variability. Random forest models achieved strong classification performance for all three taxa. The knapweed model attained near-perfect accuracy (AUC =

1.000, F1 = 0.994, Accuracy = 0.994, n = 18,106), utilizing 27 selected predictors, including Mean Diurnal Range, Temperature Seasonality, Mean Temperature of Wettest Quarter, Mean Temperature of Driest Quarter, Precipitation of Driest Month, Precipitation Seasonality, and Precipitation of Wettest Quarter, along with solar radiation in April. The midge model also performed strongly (AUC = 0.770, F1 = 0.903, Accuracy = 0.833, n = 6,970), with 24 selected features including mean temperature in January, maximum temperature in January, and precipitation in March. The wasp model showed moderate performance (AUC = 0.673, F1 = 0.700, Accuracy = 0.619, n = 3,172) based on 12 variables, including precipitation in May, precipitation in October, solar radiation in January, and solar radiation in October. These findings highlight the dominant role of thermal and precipitation seasonality, combined with solar and vapor variability, in shaping the climate niches of all three species.

Using the best-performing models for each species, we generated habitat suitability maps across North America under current and projected climate conditions using Shared Socioeconomic Pathways (SSPs), SSP1-2.6 (mild), SSP2-4.5 (moderate), and SSP5-8.5 (severe), which represent different climate trajectories based on socioeconomic variables that predict emissions. These maps quantify the probability of suitable habitat for each species on a scale from 0 to 1. Additionally, we produced climate risk maps by calculating the difference between current and future habitat suitability, identifying regions where species may face population declines or local extinctions due to climate change.

Continuous dominance maps were generated to visualize spatial patterns of herbivore-specific habitat suitability without imposing a binary 10th percentile threshold. They represent climate tolerance for each species without limiting them to areas where knapweed is present. For each climate scenario and time period combination, wasp and midge suitability rasters were first normalized to 0-1 scales by subtracting the global minimum and dividing by the range (maximum - minimum) for each species. A dominance index was then calculated as the difference between normalized wasp and normalized midge suitability values (wasp - midge), producing continuous values ranging from -1 (complete midge dominance) to +1 (complete wasp dominance), with values near zero indicating areas of co-occurrence or similar suitability without regard for magnitude of suitability.

Statistical Analysis

All analyses were conducted in R, with results considered statistically significant at $\alpha = 0.05$. We tested *H1* using linear models log(Knapweed) ~ MidgeGalls × Climate + WaspGalls × Climate + Site). Separate models tested temperature and precipitation interactions. We also generated species-specific thermal and water performance curves showing tolerance shifts in isolation versus under different species combinations using species abundance (gall densities and percent cover knapweed). We assessed species performance under different herbivore treatments (both insects, wasp only, midge only, or control) across temperature and precipitation gradients. To assess biocontrol efficacy, we implemented a blocked design, analyzing paired control and treatment plots within sites to isolate treatment effects while controlling for site-specific environmental factors. We fitted a linear model with knapweed log abundance as the response variable and treatment and site as predictors: log(Knapweed) ~ Treatment + Site.

We used structural equation modeling (SEM) to test competing hypotheses about climate-induced impacts on mechanisms driving plant-insect interactions (*H2*). Specifically, we tested whether herbivore performance was resource-limited (where knapweed abundance increases herbivore performance) or defense-limited (where knapweed abundance decreases herbivore performance) for each species. To avoid circularity, we constructed a time-lagged model where environmental stress affects knapweed abundance at time *t,* which influences insect performance at time *t*+1. The direct effects of environmental stress on insect performance were also considered. The model was fitted using maximum likelihood estimation.

Chi-square tests of independence were used to evaluate whether habitat distributions differed significantly between 2050 and 2100 time periods for both escape risk categories and herbivore habitat suitability (*H3*). Data were categorized into three levels: wasp-only, midge-only, or both species present. Standardized residuals were calculated to identify which categories contributed most to significant chi-square statistics, with absolute values >2 indicating significant deviations from expected frequencies. Geographic separation between wasp and midge habitats was quantified by comparing habitat variance and fold-change derived from dominance maps for each time period and climate intensity combination. Habitat variance was calculated as the variance in wasp versus midge suitability across all pixels, with higher variance indicating greater spatial segregation between species. Fold-change calculations were used to assess the relative magnitude of divergence between climate severity groups.

For midge-wasp interactions (*H4*), we fitted models testing whether midge performance in the presence versus absence of wasps changes across temperature and precipitation gradients: log(MidgeGalls) ~ WaspContext × Climate + Site for each insect species, and vice versa for wasps. We used ‘emmeans’ (Lenth 2024) to examine pairwise treatment comparisons at low, medium, and high climate values, revealing how interaction strengths shift with environmental stress.

Results

Both the gall midge and gall wasp respond to climate in markedly different ways. Herbivory intensity significantly interacted with climate change to affect knapweed performance, supporting *H1*. Midge gall abundance showed significant interaction with both temperature (F = 14.24, $p < 0.001$, $\beta = -0.0013$) and precipitation (F = 14.04, $p < 0.001$, $\beta = -0.0008$), indicating suppression of knapweed weakens under higher temperature and precipitation. Wasps showed no temperature interaction, but a strong response to precipitation (F = 126.22, $p < 2e\text{-}16$, $\beta = -0.0032$), with wasp suppression of knapweed abundance weakening substantially under high precipitation. These results indicate that knapweed is suppressed by the combination of herbivory and climate stress, such that biocontrol efficacy is substantially weakened under environmental conditions that promote plant growth (high temperature and precipitation).

Midges alone exhibit complex nonlinear relationships with annual mean temperature and precipitation, showing optimal performance at moderate temperatures and reduced abundance under moderate-high precipitation (Figure 16). However, when wasps are present, midges exhibit stable, high abundance across precipitation gradients, indicating facilitation under stressful conditions consistent with the SGH.

Wasps show increased abundance across temperature gradients when midges are present compared to alone, especially at high annual mean temperatures (13 °C) where both species exhibit strong performance with little variance. Additionally, wasps display distinct patterns of competition and facilitation with midges under different precipitation conditions, compared to stable abundance when solitary. Under drought conditions (250 mm), wasps exhibit substantially reduced fitness with midges present, whereas at intermediate (325 mm) and high (420 mm) precipitation, they experience facilitation. Interaction models for precipitation and temperature show opposite patterns between species.

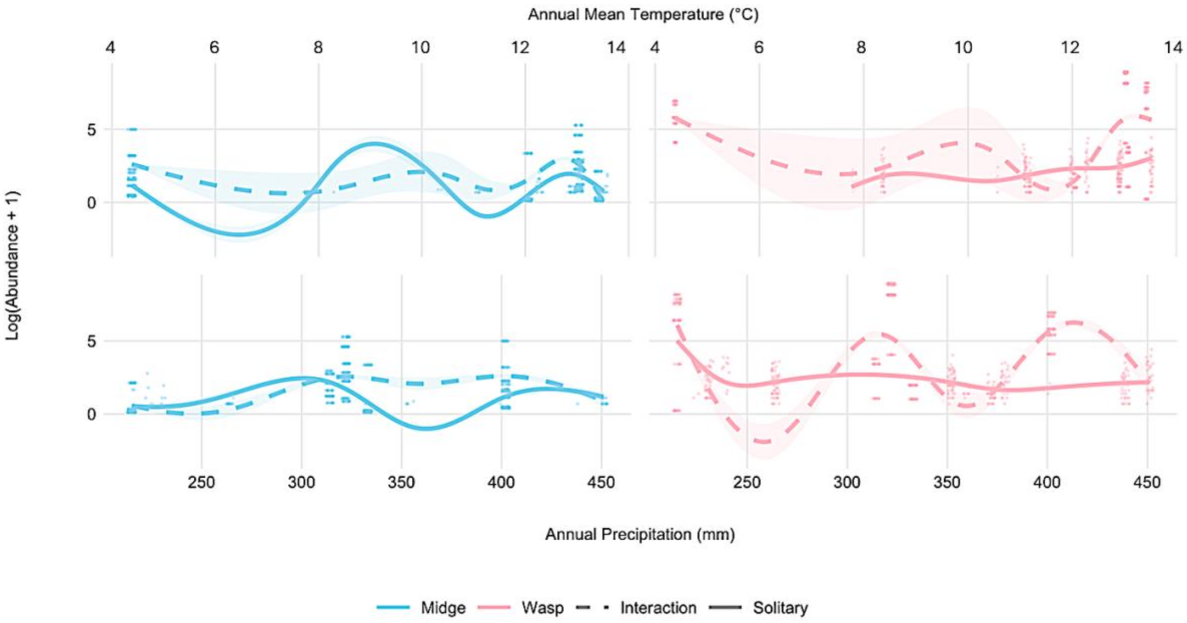


Figure 16. Thermal and water performance curves reflecting log gall abundance when the midge (blue) and wasp (pink) are alone (solid line) versus together (dashed line).

Overall, we find support for context-dependent facilitation and competition across thermal and precipitation gradients. Thermal curves indicate midges and wasps respond positively across temperature gradients only when both are present, indicating that facilitative relationships between species ameliorate thermal stress. Conversely, interaction models for precipitation show opposite patterns between species. At low precipitation (250 mm), wasps exhibit reduced abundance relative to solitary conditions, indicating competitive interactions.

Direct comparisons of interaction effects between insects across temperature and precipitation gradients fit linear models across conditions, except for midge precipitation responses (Figure 17). Both insects show negative interaction effects with increasing temperature, with wasps experiencing competition from annual mean temperatures above 10.5

°C. Meanwhile, midges appear less sensitive to thermal competition, reaching baseline solitary abundances at maximum temperature without crossing into competition. These findings fail to support the SGH regarding abiotic stress. Instead, insects facilitate each other under favorable conditions but display neutral to competitive interactions at extremes, consistent with our finding (Chapter 2) that insects exhibit plant-mediated facilitation under favorable conditions.

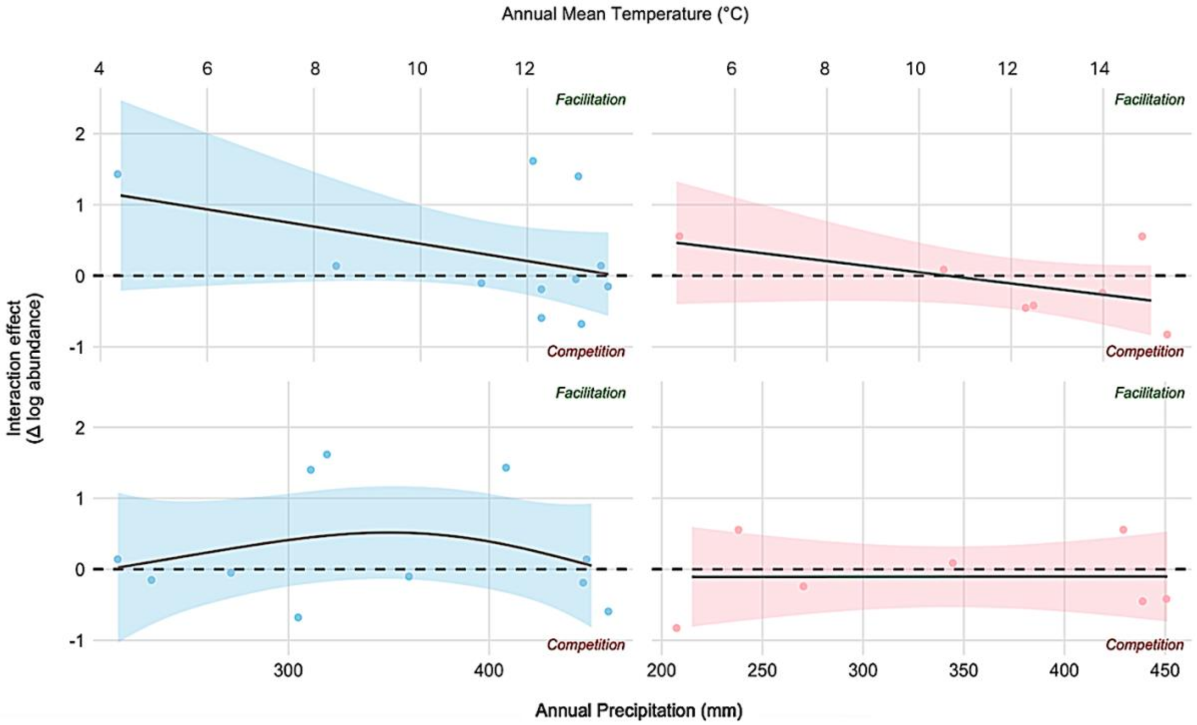


Figure 17. Models depict the change in log abundance when insects interact compared to when they are solitary. Positive responses represent facilitation, negative represent competition. The dashed line indicates neutral interactions.

Knapweed performance exhibits non-linear responses to different combinations of herbivory (Figure 18). Midges induce stronger climate-dependent effects, substantially reducing abundance at low annual mean temperatures (5 °C), but increasing it at low-moderate annual temperature (7.5 °C). At first, this may appear counterintuitive; however, these findings are consistent with compensatory growth where herbivore-induced stress shifts plant investment from belowground to aboveground growth (Hilbert *et al.* 1981; McNaughton 1983).

For both temperature and precipitation, knapweed abundance is inverted for midges compared to other treatments, supporting our hypothesis that midges are resource-limited. Despite midges' strong effect on knapweed abundance, when combined with wasps, their impact disappears, suggesting interaction effects between insects mediate the effect of midges on knapweed. The variance in the combined treatment closely mirrors the degree of divergence between midge-only and wasp-only treatments: where the two species show similar responses (low divergence), the combined treatment exhibits low variance and tracks the solitary curve, but where midge and wasp treatments diverge substantially, the combined treatment shows correspondingly high variance. This suggests that averaging across the two species obscures rather than reveals the true magnitude of their individual impacts.

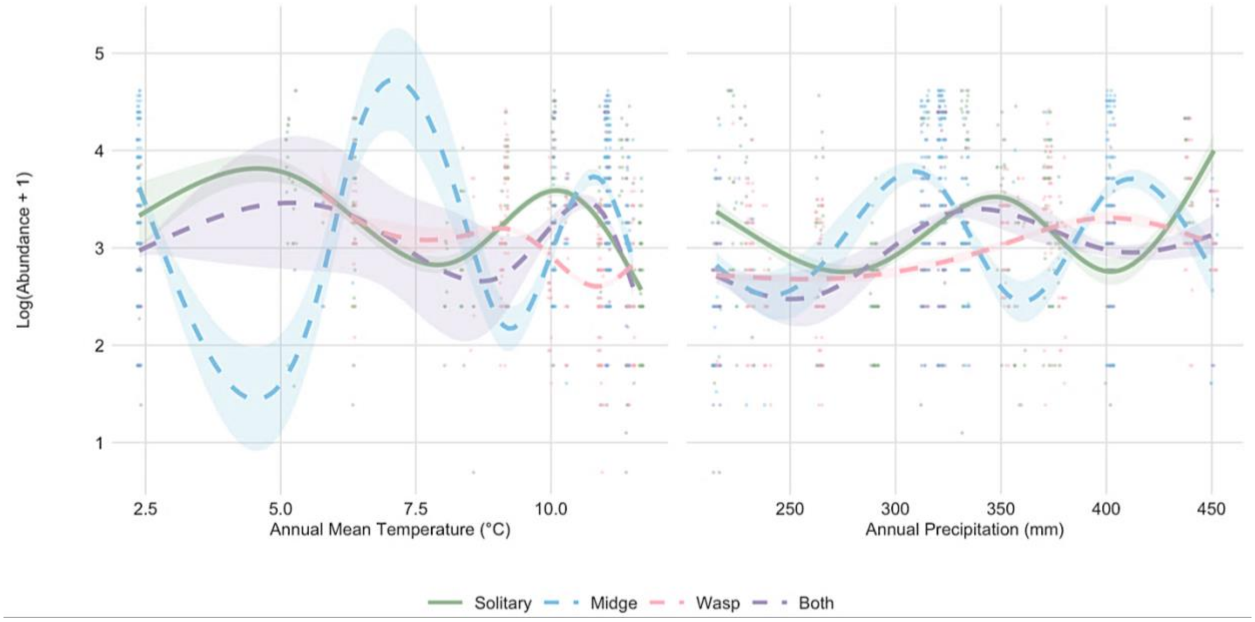


Figure 18. Knapweed performance across biocontrol contexts. Log abundance of knapweed plotted against annual mean temperature (°C) and annual precipitation (mm) under four herbivore treatments: solitary (green), midge-only (blue), wasp-only (pink), and both insects (purple). Note the wasp treatment is absent below 5 °C.

We also tested whether climate stress mediated herbivore-herbivore interactions (H4), finding strong support for environmentally-mediated facilitation under stressful conditions ($p < 0.010$). Exmaining main effects confirms that climate conditions were stressful for both species: midge abundance decreased with temperature ($\beta = -0.148$, -14% per °C) and precipitation ($\beta = -0.006$, -0.6% per mm), while wasp abundance declined under increased temperature ($\beta = -0.098$, 9% per °C) and precipitation ($\beta = -0.125$, -12% per mm).

Midge facilitation by wasps increased with temperature ($\beta = +0.100$, + 11% per °C, $p <$ 1e-07) and weakly with precipitation ($\beta = +0.026$, +3% per mm, $p = 0.006$). Wasp facilitation by midges showed even stronger climate dependencies, increasing substantially with temperature ($\beta = +0.271$, 31% per °C, $p <$ 1e-08) and precipitation ($\beta = +0.100$, +11% per mm, $p <$ 1e-14). Our models also controlled for the effect of knapweed abundance, indicating that interaction effects were not driven by differences in host plants. Interestingly, this conflicts with interaction trends observed in precipitation performance curves (Figure 16), suggesting that increased knapweed abundance at higher annual mean precipitation values promotes competition between herbivores. Together, these findings support the hypothesis that interactions between the midge and wasp conform to the SGH, with stressful environmental conditions promoting facilitation.

Overall, we observed significant treatment effects on knapweed abundance ($F = 247.9$, $p < 0.001$), with insects suppressing aboveground growth in all treatment combinations (Figure 19). Despite knapweed abundance appearing stable between the knapweed-only and dual-herbivore treatment depicted in Figure 18, analysis controlling for environmental variation by comparing paired control-treatment plots within sites confirms that averaging across species obscured the true effect of dual-insect treatments. Midge-only treatment reduced knapweed abundance 7.4%, wasp-only treatment reduced abundance by 13.2%, and both insects together produced the strongest suppression at 30.7% reduction. These findings indicate synergistic effects between biocontrol insects, strongly suggesting biological control programs should release both insects together to achieve greater knapweed suppression. The midge-only effect size may be underestimated due to context-dependent responses where midges alternately suppress or enhance knapweed aboveground growth across environmental gradients (Figure 18), causing positive and negative effects to partially cancel out in the overall analysis. However, belowground growth was not measured, it is not possible to determine the true extent of midge impact via compensatory growth (Wise & Abrahamson 2008).

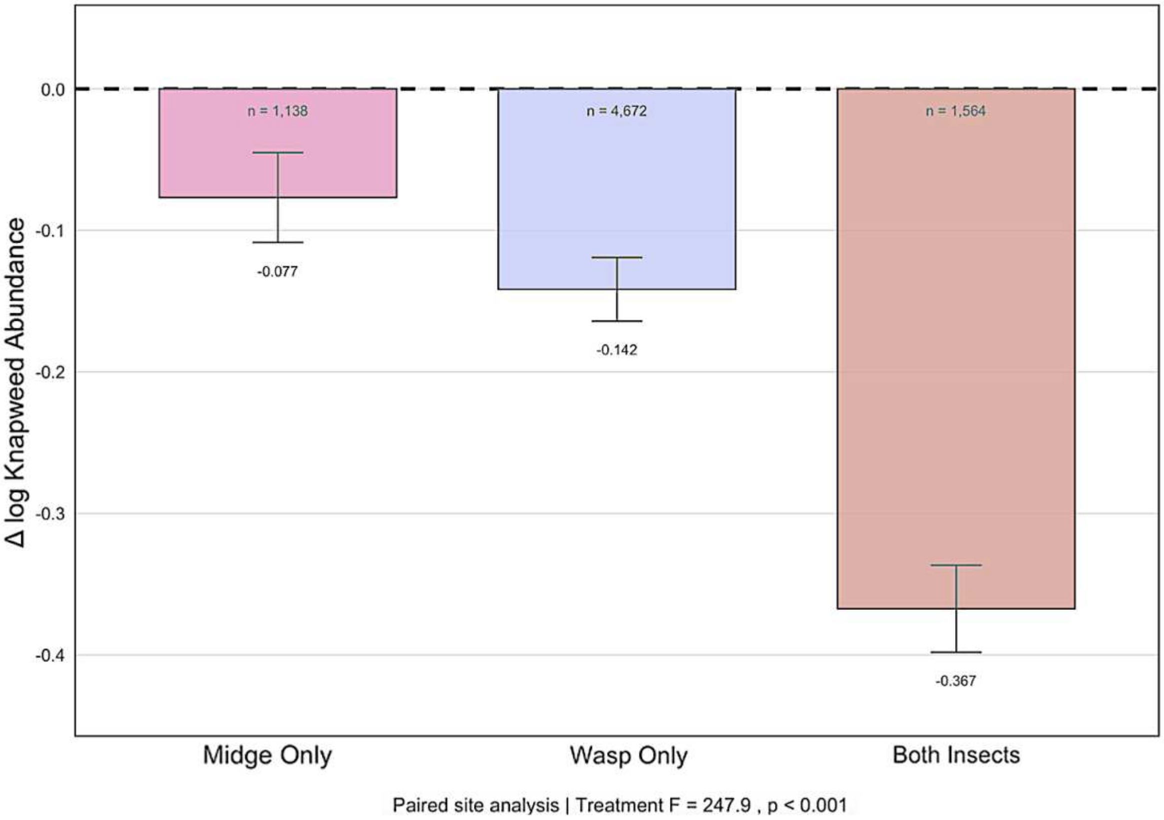


Figure 19. Biocontrol efficacy on knapweed abundance. Change in log knapweed abundance relative to control plots across midge-only (pink), wasp-only (blue), and both insects (orange) herbivore treatments.

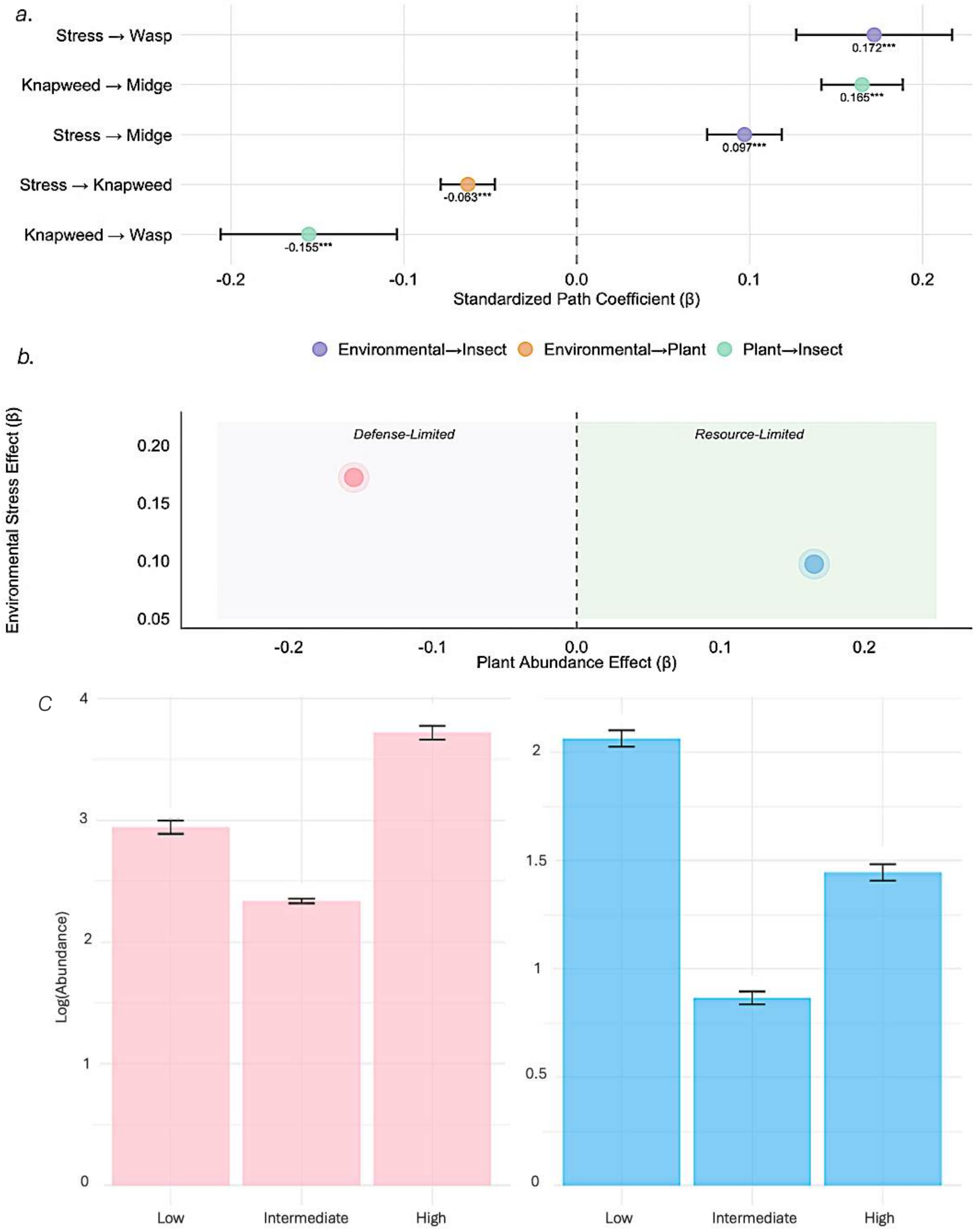


Figure 20. Structural equation modeling reveals distinct ecological strategies between the wasp (pink) and midge (blue). Environmental conditions selected from the knapweed distribution model represent conditions resulting in plant stress as reflected by knapweed abundance, where low = high abundance, low stress; intermediate = intermediate levels of abundance and stress; high = low abundance, high stress. **(a)** Standardized path coefficients showing environmental and

plant-mediated effects on herbivore performance, with pathway types color-coded. **(b)** Defense versus resource limitation framework plotting environmental stress effects against plant abundance effects for each species. Pathways represent driver → receiver relationships, with negative values indicating reduced performance and positive values indicating enhanced performance. **(c)** Species performance on knapweed across environmental stress gradients, demonstrating differential responses between wasps and midges.

SEM and standardized path coefficients confirm climate-induced changes in plant fitness produce species-specific insect responses, supporting hypothesis H2 (Figure 20*a,c*). The defense versus resource limitation framework reveals contrasting ecological strategies: wasps benefit from reduced plant defenses under increased abiotic stress, whereas midges benefit from greater resource availability (knapweed abundance; Figure 20b). Additionally, both insects exhibit increased abundance in response to direct environmental stress, suggesting low transferability of climate models between species (Figure 20a). Of note, our model isolates the effects of knapweed abundance on insect herbivores from the effect of herbivores on knapweed abundance by incorporating time lags that separate cause and effect.

Mapping

Contemporary climate suitability models reveal share substantial overlap in environmental niche space for *R. repens, J. ivannikovi,* and *A. acroptilonica* across North America. Both biocontrol agents average 55–56% suitability, indicating moderate climatic fit. Knapweed exhibits greater climate tolerances than its herbivores, reflected by higher average suitability (u = 66.3%) and broader range (Figure 21). Thus, under current climate conditions, knapweed is able to escape its biological control insects in some contexts.

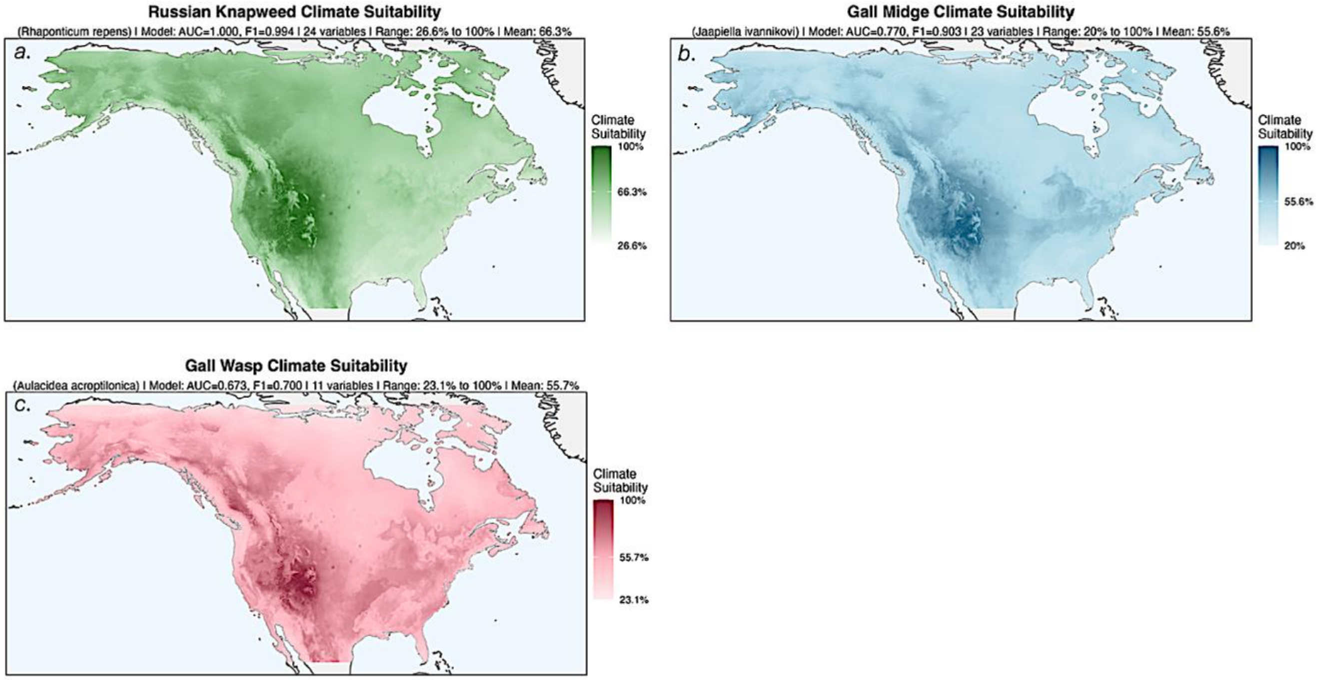


Figure 21. Climate envelopes for **(a)** *R. repens* (green), **(b)**. *J. ivannikovi* (blue), and **(c)**. *A. acroptilonica* (pink) under current conditions. Darker colors represent greater climate suitability.

Our climate projections revealed substantial shifts in biocontrol agent habitat suitability under increasing environmental stress (Figure 22). Areas suitable for midge-only establishment show consistent declines, exhibiting a 19% reduction (2.7 million km^2 to 2.2 million km^2) from mild to severe conditions. Similarly, wasp-only suitable habitat contracts from 1.4 million km^2 to 1.0 million km^2, representing a 29% decline.

Notably, at the state-level this pattern predicts range expansions into high-elevation montane environments (Figure 23), reflecting consistent environmental shifts in knapweed range operating at both local and continental scales.

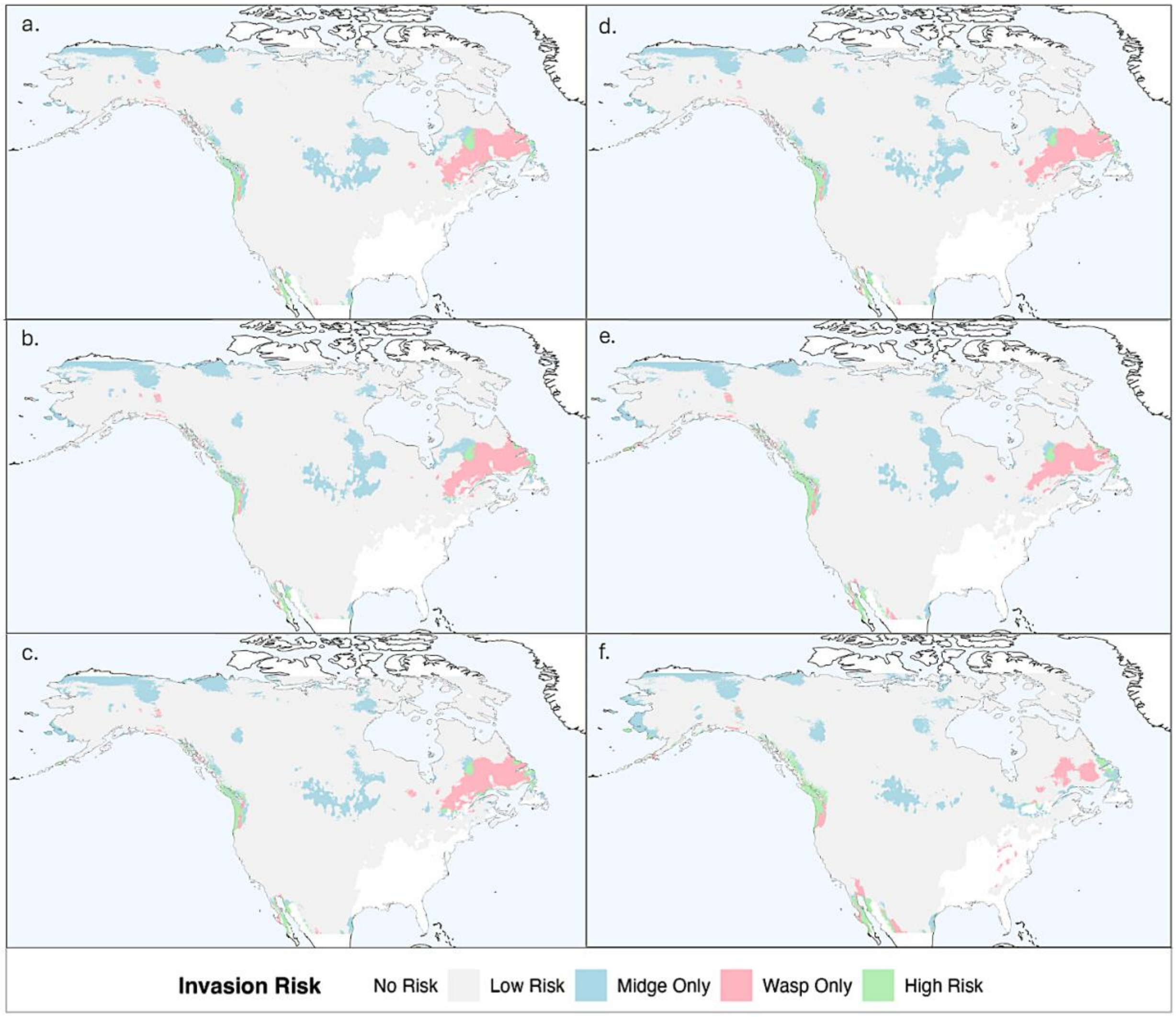


Figure 22. Projected invasion risk of *R. repens* across North America under future climate scenarios for the years 2050 (left column: a–c) and 2100 (right column: d–f). Rows represent increasing climate forcing scenarios based on Shared Socioeconomic Pathways (SSPs): SSP1-2.6 (mild; a, d), SSP2-4.5 (moderate; b, e), and SSP5-8.5 (severe; c, f). Invasion risk is categorized by biocontrol match: gray = both agents effective, blue = midge only present, pink = wasp escape only, green = complete escape (knapweed suitable, no agents).

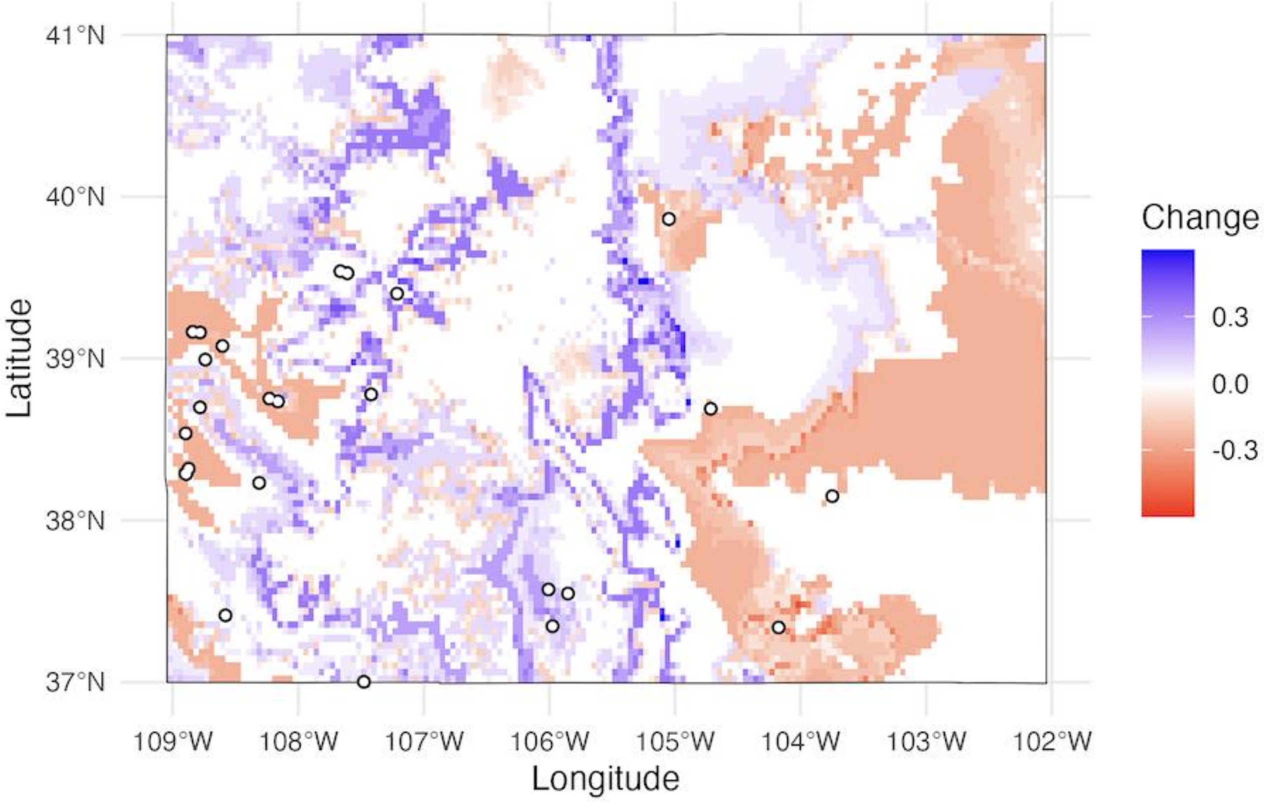


Figure 23. *R. repens* environmental suitability for the year 2050 in Colorado. Red depicts areas where knapweed is predicted to decrease while blue areas represent anticipated increases relative to current distributions. White points represent field sites used for predictions.

Climate suitability analysis revealed striking differences in niche breath between herbivores, supporting our hypothesis (H3) that response to climate change is species-specific (Figure 24). Midges demonstrated substantially broader climate tolerance (ranging from 2.7 million km² to 2.4 million km² from mild to severe scenarios) relative to wasps (from 1.4 million km² to 1 million km² from mild to severe scenarios). This represents a 2:1 ratio in niche breath in 2050 under mild climate projections increasing to a 3:1 ratio in 2100 under severe conditions, favoring the midge across all conditions.

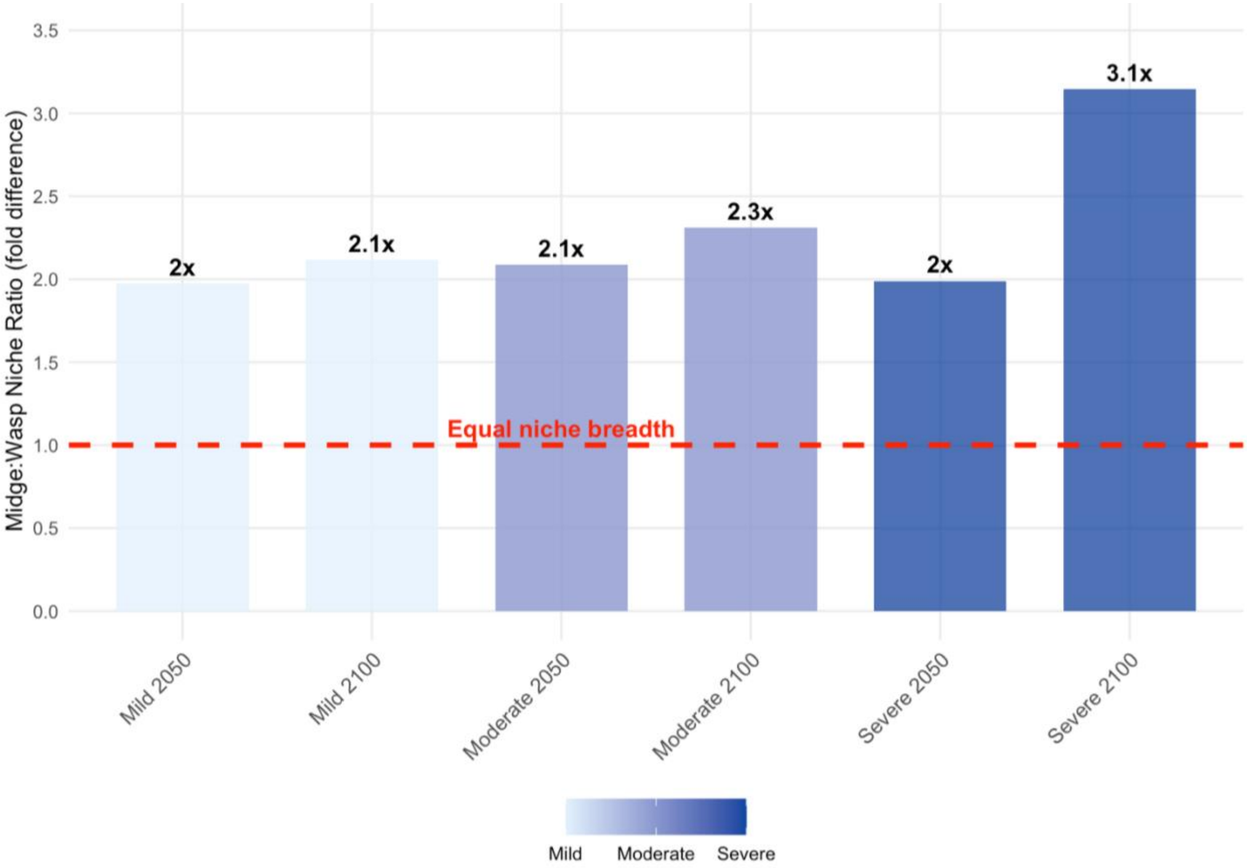


Figure 24. Climate niche breath of the midge and wasp. Climate severity increases from light to dark blue. The x-axis represents each unique climate-year combination tested while the y-axis represents the ratio of suitable habitat for midges relative to wasps with values >1 representing greater climate tolerance in the midge, values < 1 representing greater tolerance in the wasp and values = 0 representing equal climate niche breath between insects, marked by the dashed red line.

Although both insects exhibit range contractions from 2050 to 2100 under increasing climate severity when constrained by knapweed presence (Figure 25a), their *possible* ranges based solely on climate tolerance (Figure 25b) reveal distinctly different patterns. For midges, response to climate change reverses direction, exhibiting range *expansions* of over 350% under severe climate scenarios ($\chi^2 = 63.42$, $df = 2$, $p = 1.69e\text{-}14$), indicating that host presence rather than environmental stress limits midge geographic range in future scenarios. Wasps show consistent range contraction between their host-restricted and unrestricted (-250%) distributions, reflecting that they are not substantially constrained by host availability. Comparing the area that both insects are present reveals dramatic differences, expanding by only 3% under severe climate when constrained to knapweed-suitable habitat compared to a 700% increase when limited only by climate ($\chi^2 = 361.62$, $df = 2$, $p < 2.2e\text{-}16$). Importantly, this indicates that wasp-only range contractions are associated with midge-only expansions. Thus, rather than indicating poor environmental tolerance by wasps, this reflects that under severe climate scenarios, the midge climate niche becomes more similar to that of wasps, leading to niche convergence. This supports our prediction that herbivores would exhibit distinct, species-specific responses to climate change (H3-I), but contradicts our prediction (H3-II) that increasing climate severity would reduce spatial overlap between herbivores. This niche convergence under severe climate scenarios demonstrates that environmental stress creates feedback loops that alter the spatial context of species interactions by shifting populations from geographic separation toward increased co-occurrence.

However, species dominance maps that consider habitat suitability rather than binary presence/absence reveal that the relationship between climate and herbivore coupling is more nuanced than it first appears to be (Figure 26). Geographic separation metrics provided strong support for our third hypothesis, demonstrating that climate change drives divergence of herbivore optimal habitat. Under severe climate scenarios, habitat variance increased by 42.4% from 2050 to 2100, representing a 6.6-fold greater increase than under mild scenarios (6.4% increase). The stark increase in divergence under severe climate scenarios indicates that climate-driven range divergence increases non-linearly with environmental stress.

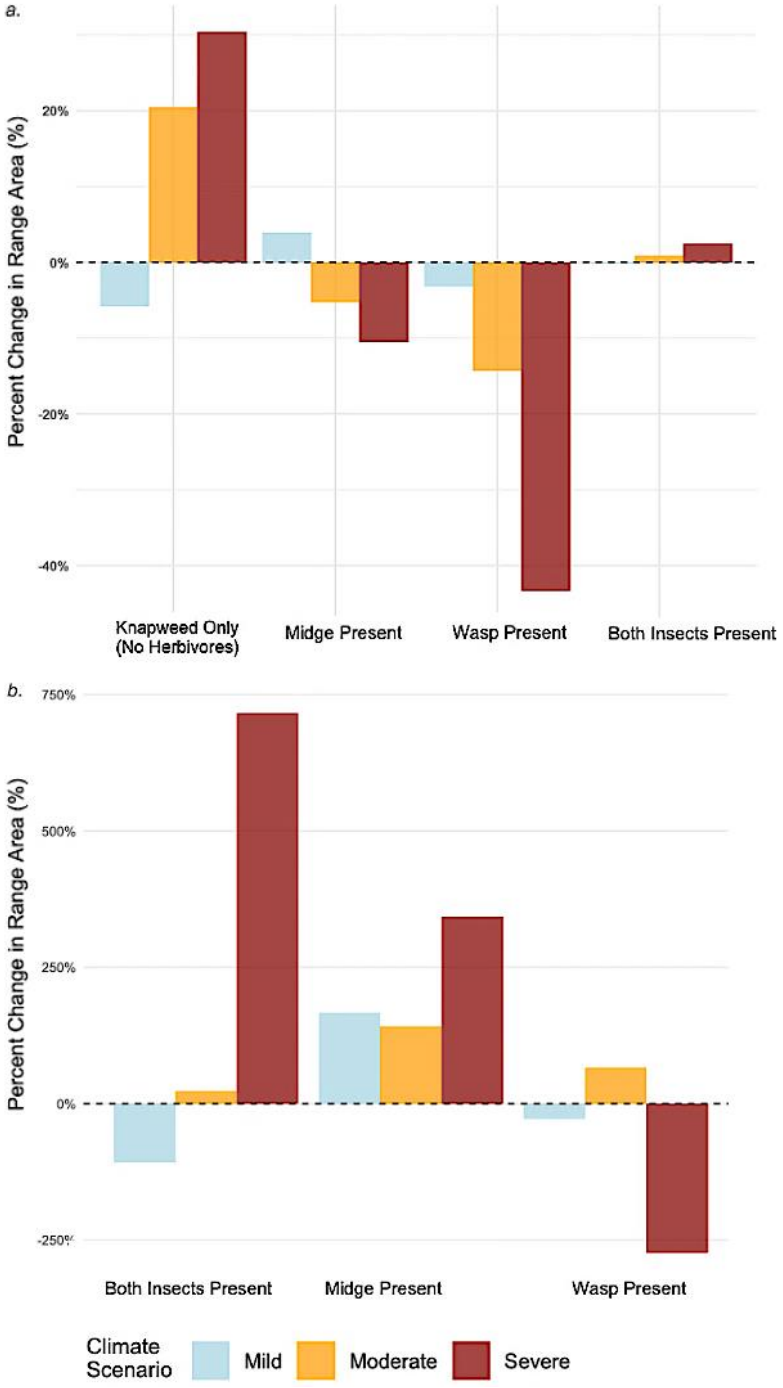


Figure 25. Percent change in species range areas from 2050 to 2100 under different climate change scenarios, including mild (blue), moderate (yellow), and severe (red). Bars represent percent change in suitable habitat area between mid-century (2050) and late-century (2100) projections. Positive values indicate range expansion, while negative values indicate range contraction. Dashed line represents no change between time periods. **(a-b)** Herbivore combinations include both insects, midge-only, wasp-only and **(a)** knapweed only. Panels are habitat overlap with knapweed and **(b)** climate tolerances regardless of knapweed presence.

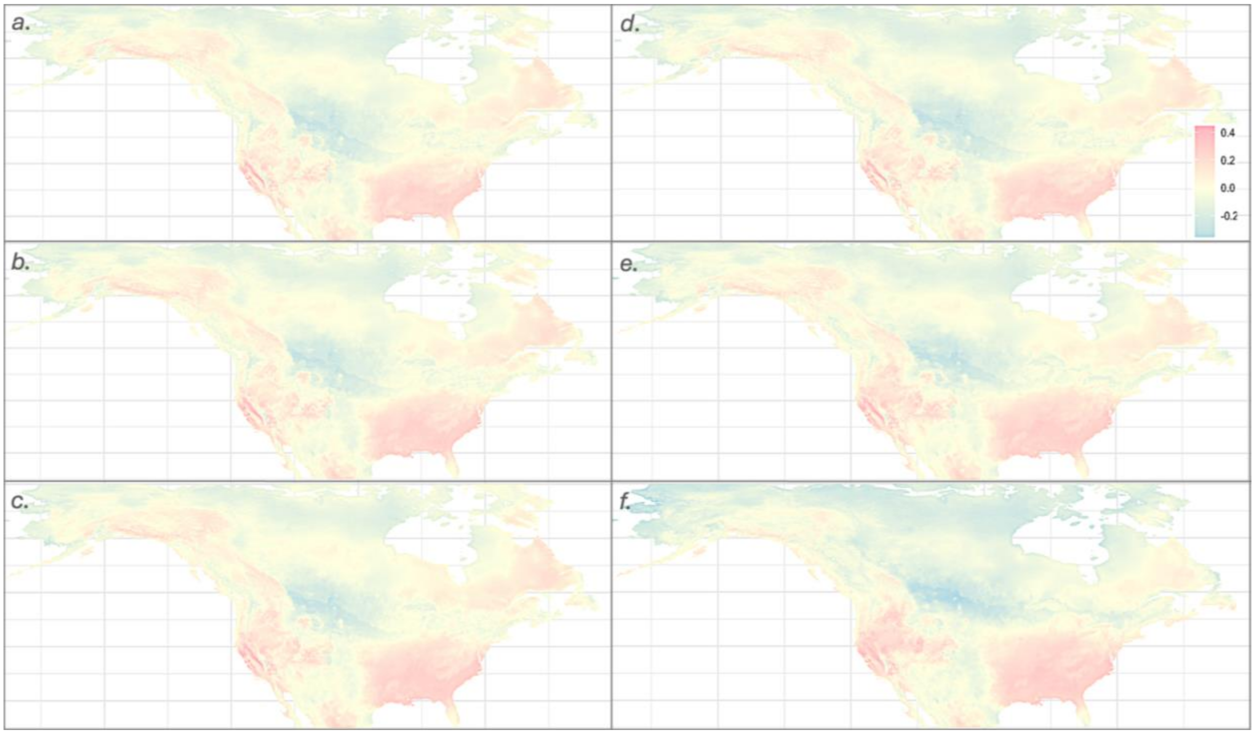


Figure 26. Species dominance maps showing spatial patterns of herbivore habitat suitability across North America. Maps display dominance indices ranging from midge-dominated areas (blue) through co-occurrence zones (yellow) to wasp-dominated areas (pink). Panels show: **(a)** 2050 mild climate, **(b)** 2050 moderate climate, **(c)** 2050 severe climate, **(d)** 2100 mild climate, **(e)** 2100 moderate climate, and **(f)** 2100 severe climate scenarios.

Discussion

Our findings reveal complex, context-dependent responses to climate change across a highly coevolved plant-herbivore network, challenging traditional predictions about the vulnerability of specialist networks to environmental stress (Kadoya & McCann 2015; Koch *et al*. 2024). We demonstrate support for environmentally modulated plant-insect and plant-mediated insect interactions from the community to landscape scale, revealing these interactions are highly context-dependent. Although individual herbivore performance declined under climate stress (supporting predictions for specialist species; Clavel *et al*. 2011), they exhibited increasingly facilitative interactions when present together, consistent with the Stress Gradient

Hypothesis (Bertness & Callaway 1994), lending support to the small but growing number of studies to document the SGH in interactions between herbivores. However, without controlling for knapweed abundance, the same relationship appears competitive, reflecting how feedbacks between abiotic and biotic stressors shift interactions.

Moreover, our finding that biocontrol efficacy decreased under conditions favoring plant growth (high temperature and precipitation), suggests that environmental stress plays a critical role in shaping plant resilience to herbivory (González-Tokman *et al*. 2020). Together, these findings implicate plant defense signaling as an important mediator of network response to environmental stress. This yields important insights into understanding the impacts of global change (Facon *et al*. 2006), highlighting that interactions between species may be more important than absolute climate tolerances in predicting how communities will respond to environmental stress (He *et al*. 2013). For instance, when we isolated the time-lagged effects of knapweed abundance on herbivore performance, we found species-specific responses with the wasp demonstrating a defense-limited phenotype while the midge exhibited resource limitation.

At the landscape scale, climate change may reduce *or* enhance the efficacy of biological control agents (Phillips *et al*. 2008), depending on the direction of herbivore-herbivore interactions. This uncertainty emphasizes the importance of research that considers multi-species interactions and their responses to environmental change. This is particularly true for biological control programs, where inadvertently introducing competitors together may compromise biocontrol efficacy, while introducing cooperative species alone may limit the effect size and applicability of agents.

Our geographic projections revealed an unexpected pattern of niche convergence under severe climate scenarios, where midges and wasps shifted from spatial separation toward increased co-occurrence, demonstrating that connection strength and shared evolutionary history may ameliorate the effects of network sparsity on community resilience to climate change. This challenges the perception that specialist networks with sparse connections are inherently more vulnerable to collapse (Biggs *et al*. 2020), suggesting that characteristics such as the direction and magnitude of individual connections may be more important than connection quantity in determining resilience.

The distinct climate sensitivities observed between species—with wasps being more sensitive to precipitation, midges more sensitive to temperature, and knapweed benefiting from broth increased temperature and precipitation—appeared to drive species-specific habitat suitability differences before populations converged under the most extreme climate scenario. Interestingly, even as herbivore populations showed increased convergence by land area (km^2), they simultaneously exhibited more extreme distributions within shared habitat, with relatively few area of high suitability and many areas of low suitability (Figure 26). These results reveal a paradoxical pattern wherein climate change simultaneously drives both convergence and divergence of herbivore distributions, reflecting microhabitat specialization amidst macrohabitat generalization.

As expected, the future realized ranges—where insect environmental tolerances overlap with knapweed presence—of both species decreased with increasing climate severity. However, when considering *theoretical* ranges, contrary to expectations, midges exhibited sizable range expansions. Additionally, the broad climate tolerance exhibited by midges conforms to theoretical expectations that *r*-selected species exhibit greater capacities for adapting to environmental stressors through phenotypic plasticity and/or rapid adaptation (Albaladejo-Robles *et al*. 2023). Beyond its *r*-selected life history characteristics, the midge’s recent introduction to North America and history of multiple releases from genetically distinct populations both contribute to elevated genetic diversity and thus increase the potential for rapid selection to novel climate conditions.

This also highlights an opportunity for strategic biocontrol management. To promote adaptive responses to novel environmental conditions, conducting regular supplemental releases using insects from genetically diverse source populations can maintain existing genetic variation and introduce new genetic variation (Phillips *et al*. 2008). Additional insights come from the synergistic effects of insect interactions on biocontrol efficacy (30.7% knapweed suppression with both species versus 13.2% and 7.4% for the wasp and midge alone, respectively), suggesting that multi-species releases should be prioritized to improve biocontrol efficacy and increase stress tolerance via facilitation (Denoth *et al*. 2002).

However, range contractions of the wasp, increasingly extreme distributions of both species within their suitable ranges, and reduced biocontrol efficacy of single-insect treatments under climate change suggests these agents, particularly the wasp, could become less reliable as climate change intensifies. This is particularly pertinent given evidence for rapid climate-induced evolution in *R. repens* populations (Chapter 3), increasing its potential to escape its herbivore enemies (Keane & Crawley 2002). These findings call for integrated management approaches that consider multitrophic feedback loops when studying global change effects (Dyer & Letourneau 2013). They also highlight the importance of network connectivity in determining resilience to environmental stressors (Biggs *et al*. 2020).

Future research should develop analytical frameworks that incorporate empirical data on direct and indirect species interactions, phenotypic plasticity responses, and evolutionary adaptation rates into bioclimate projection models. Additionally, the gall midge *J. ivannikovi*, should be investigated further to determine if it exhibits signs of evolutionary adaptation since being introduced to North America. Not only would this knowledge inform biological control efforts, but it also adds to the theoretical value of the knapweed-biocontrol network as a model for eco-evolutionary changes to network dynamics. Understanding the factors mediating resilience in specialist networks is a promising research avenue, particularly in biological control systems which may be uniquely well-suited to study rapid eco-evolutionary dynamics, given their known introduction and evolutionary histories, and examine network resilience given their simplified community structures. Finally, the plant-mediated versus plant-excluded interaction pathways we identified warrant further investigation, especially regarding the role of compensatory growth and plant defense signaling in mediating species interactions under varying environmental conditions.

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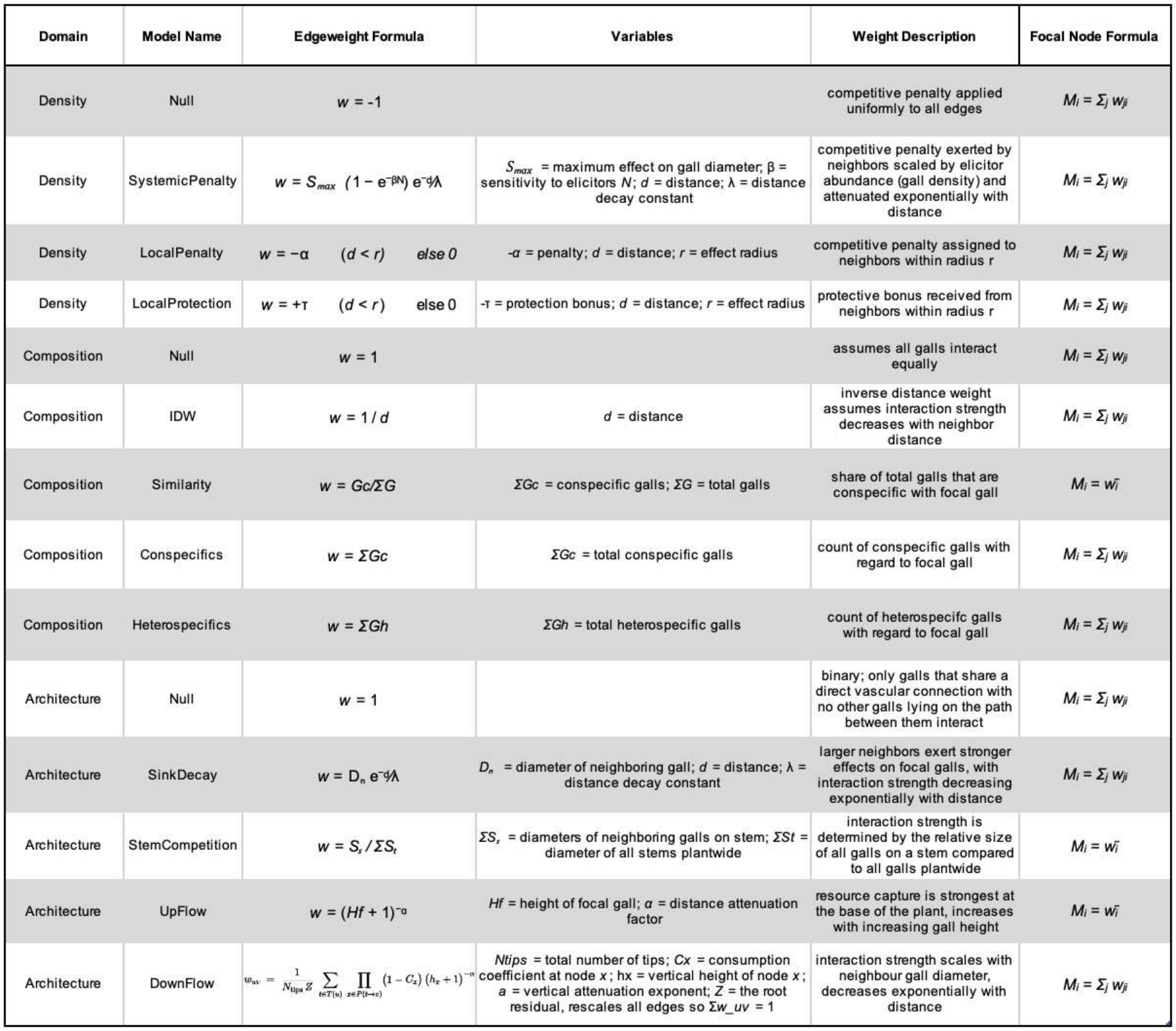
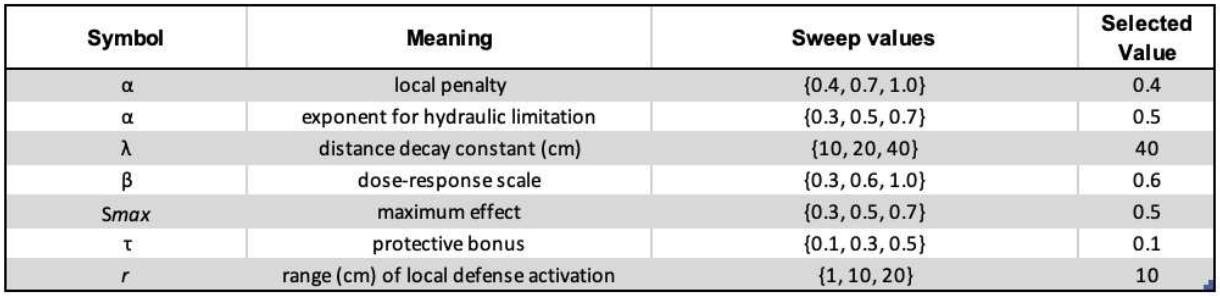
Appendix

Appendix Table 1. Summary of all candidate network models and parameter definitions. Each model's domain, edgeweight formula, variable definitions, and description are provided. Node-level metrics were calculated as either the sum (*Σjwji*) or average (*wi*) of the focal node's edgeweights.

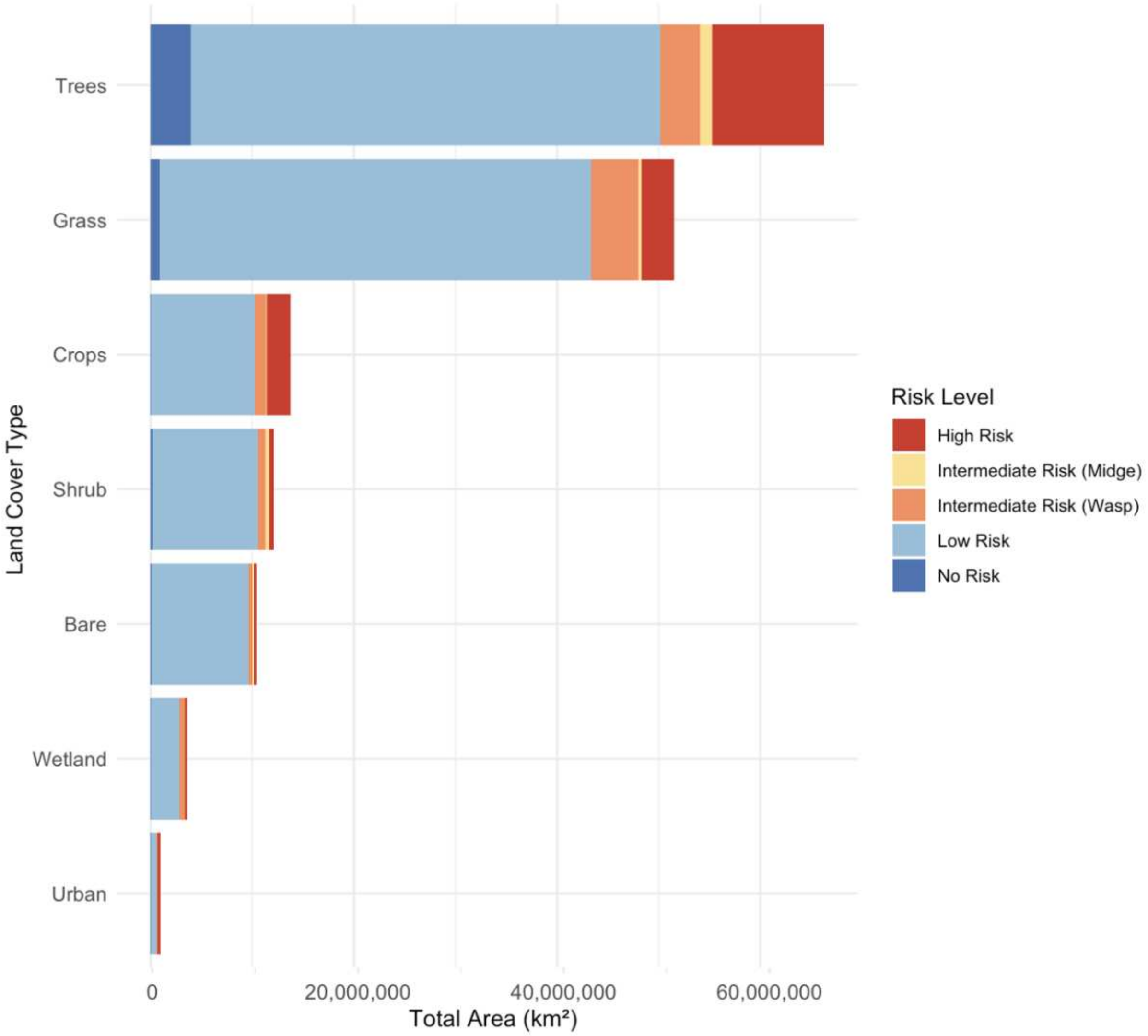


Domain	Model Name	Edgeweight Formula	Variables	Weight Description	Focal Node Formula
Density	Null	$w = -1$		competitive penalty applied uniformly to all edges	$M_i = \Sigma_j w_{ji}$
Density	SystemicPenalty	$w = S_{max}\ (1 - e^{-\beta N})\ e^{-d/\lambda}$	S_{max} = maximum effect on gall diameter; β = sensitivity to elicitors N; d = distance; λ = distance decay constant	competitive penalty exerted by neighbors scaled by elicitor abundance (gall density) and attenuated exponentially with distance	$M_i = \Sigma_j w_{ji}$
Density	LocalPenalty	$w = -\alpha \quad (d < r) \quad else\ 0$	$-\alpha$ = penalty; d = distance; r = effect radius	competitive penalty assigned to neighbors within radius r	$M_i = \Sigma_j w_{ji}$
Density	LocalProtection	$w = +\tau \quad (d < r) \quad \text{else } 0$	$-\tau$ = protection bonus; d = distance; r = effect radius	protective bonus received from neighbors within radius r	$M_i = \Sigma_j w_{ji}$
Composition	Null	$w = 1$		assumes all galls interact equally	$M_i = \Sigma_j w_{ji}$
Composition	IDW	$w = 1 / d$	d = distance	inverse distance weight assumes interaction strength decreases with neighbor distance	$M_i = \Sigma_j w_{ji}$
Composition	Similarity	$w = Gc/\Sigma G$	ΣGc = conspecific galls; ΣG = total galls	share of total galls that are conspecific with focal gall	$M_i = w_{\bar{i}}$
Composition	Conspecifics	$w = \Sigma Gc$	ΣGc = total conspecific galls	count of conspecific galls with regard to focal gall	$M_i = \Sigma_j w_{ji}$
Composition	Heterospecifics	$w = \Sigma Gh$	ΣGh = total heterospecific galls	count of heterospecifc galls with regard to focal gall	$M_i = \Sigma_j w_{ji}$
Architecture	Null	$w = 1$		binary; only galls that share a direct vascular connection with no other galls lying on the path between them interact	$M_i = \Sigma_j w_{ji}$
Architecture	SinkDecay	$w = D_n\ e^{-d/\lambda}$	D_n = diameter of neighboring gall; d = distance; λ = distance decay constant	larger neighbors exert stronger effects on focal galls, with interaction strength decreasing exponentially with distance	$M_i = \Sigma_j w_{ji}$
Architecture	StemCompetition	$w = S_s / \Sigma S_t$	ΣS_s = diameters of neighboring galls on stem; ΣSt = diameter of all stems plantwide	interaction strength is determined by the relative size of all galls on a stem compared to all galls plantwide	$M_i = w_{\bar{i}}$
Architecture	UpFlow	$w = (Hf + 1)^{-\alpha}$	Hf = height of focal gall; α = distance attenuation factor	resource capture is strongest at the base of the plant, increases with increasing gall height	$M_i = w_{\bar{i}}$
Architecture	DownFlow	$w_{uv} = \frac{1}{N_{\text{tips}} Z} \sum_{t \in T(u)} \prod_{x \in P(t \to v)} (1 - C_x)\ (h_x + 1)^{-a}$	$Ntips$ = total number of tips; Cx = consumption coefficient at node x; hx = vertical height of node x; a = vertical attenuation exponent; Z = the root residual, rescales all edges so $\Sigma w\_uv = 1$	interaction strength scales with neighbour gall diameter, decreases exponentially with distance	$M_i = \Sigma_j w_{ji}$

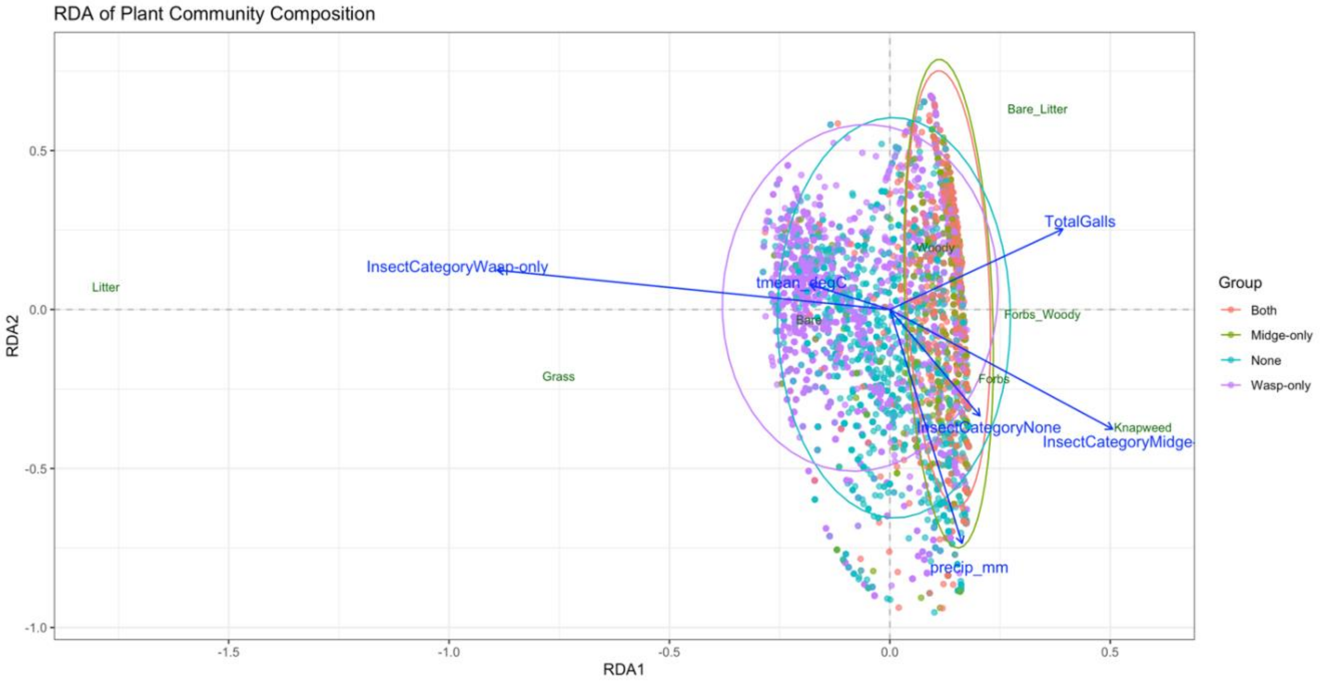
Appendix Table 2. Signal sweep values used to optimize parameters for network models.



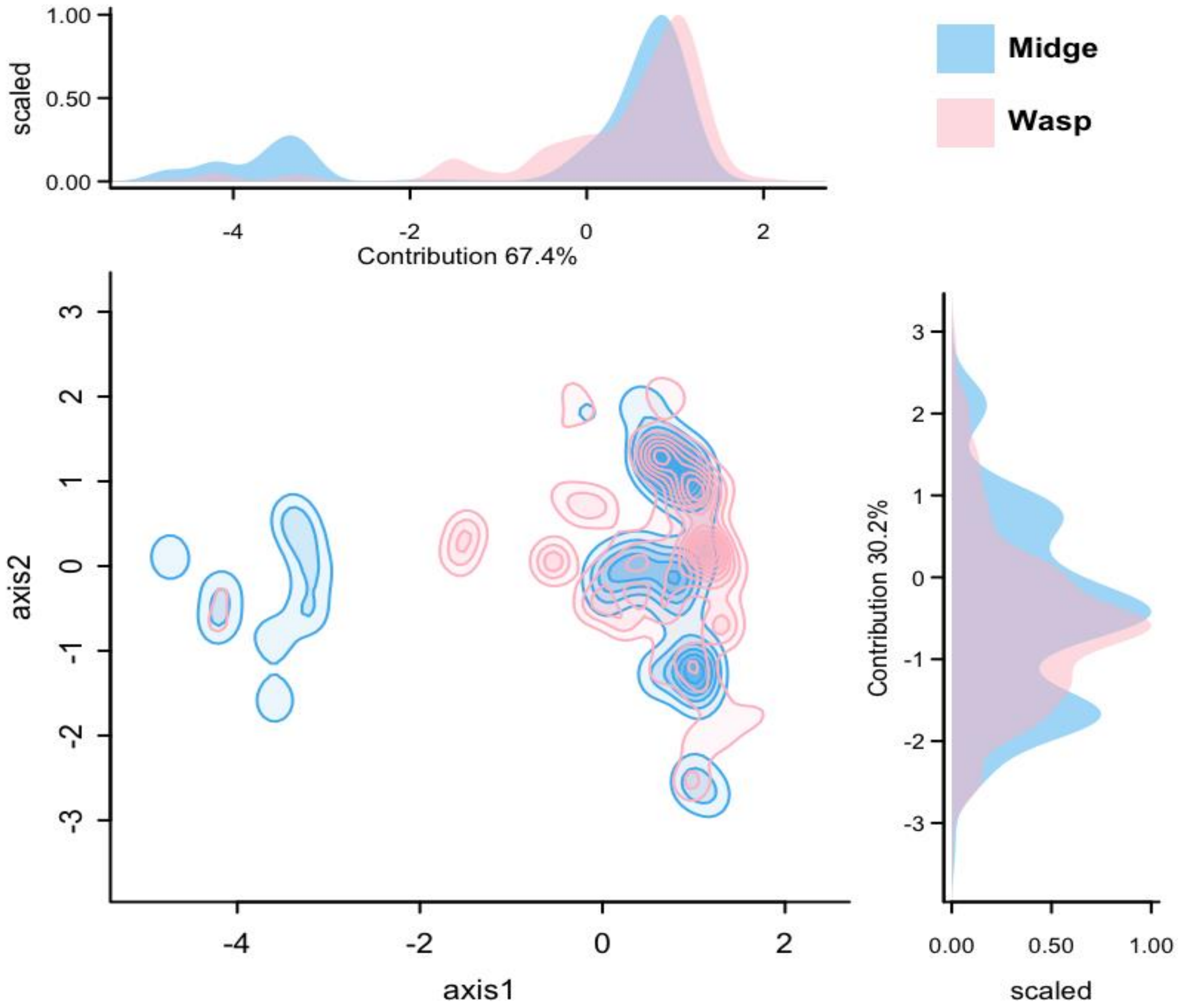
Symbol	Meaning	Sweep values	Selected Value
α	local penalty	{0.4, 0.7, 1.0}	0.4
α	exponent for hydraulic limitation	{0.3, 0.5, 0.7}	0.5
λ	distance decay constant (cm)	{10, 20, 40}	40
β	dose-response scale	{0.3, 0.6, 1.0}	0.6
S*max*	maximum effect	{0.3, 0.5, 0.7}	0.5
τ	protective bonus	{0.1, 0.3, 0.5}	0.1
r	range (cm) of local defense activation	{1, 10, 20}	10



Appendix Figure 1. Total area by land cover type at risk of invasion by knapweed based on overlap with knapweed-only (high risk), midge or wasp only (intermediate risk), both insects (low risk), or knapweed absent (no risk) areas under future projected range distributions.



Appendix Figure 2. Redundancy analysis plot depicting influences on plant community composition. RDA1 represents bioclimatic variables and RDA2 represents percent cover of knapweed versus native plants (green labels). Ellipses are 95% CI with color-coding corresponding to insect release treatment. Explanatory variables are depicted by vectors (blue arrows) with lengths proportional effect size. The position of plant categories (green text) relative to vectors indicates the direction and magnitude of correlations.



Appendix Figure 3. Climatic niche overlap between biocontrol agents. PC1 (67.4% variance) represents a temperature-precipitation gradient while PC2 (30.2% variance) captures secondary climate variation. Kernel density plots show the distribution of midge (blue) and wasp (pink) occurrence records in principal component space derived from four annual climate variables (temperature, precipitation, photosynthetically active radiation, vapor pressure). Contour lines and density curves represent gall density. Overlapping regions indicate niche overlap.