ENHANCING PEST MANAGEMENT OF ABOVE- AND BELOWGROUND HERBIVORES THROUGH PLANT-MEDIATED EFFECTS

By

Kayleigh Courard Hauri

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Entomology—Doctor of Philosophy

2024

ABSTRACT

A major challenge in plant-insect interactions is predicting the outcome of multiple herbivory, which can have drastically different effects on plants and their respective communities compared to simple additive effects. This can be especially difficult for interactions between herbivores above and belowground, which never interact directly but create a very different host environment for subsequent feeders through changes to plant nutrition and chemistry. Thus far, the variation in outcomes has made it difficult to predict how a given set of species will interact unless that combination has been previously studied; furthermore, it is often unclear how these interactions change when exposed to a wider community. We conducted a meta-analysis along with greenhouse and laboratory experiments to determine how plant-mediated interactions between above- and belowground herbivores change under a suite of biologically relevant factors. First, we determined what the overall outcomes were for interactions between aboveground insect herbivores and belowground plant feeding nematodes, and how these interactions affected chewing insect growth, phloem-feeding insect reproduction, and nematode reproduction, as well as carbon and nitrogen location within a plant. Second, we used laboratory and greenhouse experiments to investigate how constitutive plant defense altered the plant-mediated interaction between a chewing herbivore (Colorado potato beetle, *Leptinotarsa decemlineata*) and the Northern root-knot nematode (*Meloidogyne hapla*). Finally, we investigated how belowground damage by the Western corn rootworm (*Diabrotica virgifera virgifera*) influenced population growth of corn aphids (*Rhopalosiphum maidis*) as well as the preference and performance of a generalist lady beetle (*Hippodamia convergens*) and an aphid parasitoid (*Aphidius colemani*), as well as aboveground plant volatiles. We found that foliar chewing insect growth was decreased in the presence of gall nematodes and increased in the

presence of cyst nematodes, and that concurrent feeding by plant feeding nematodes and aboveground insect herbivores alters the distribution of carbon and nitrogen in the plant. Next, we found that constitutive level of plant defense can alter the directionality and strength of interactions between nematodes and foliar chewing herbivores. Lastly, we determined that feeding by a belowground chewing herbivore can indirectly affect foliar aphid reproduction as well as the third trophic level through reproductive effects on aphids and aboveground plant volatiles, but that these effects are change over time and affect a predator and parasitoid differently. This work fills in several gaps in our existing framework of plant-mediated interactions and allows us to fine-tune predictions about which focal systems will be most susceptible to plant-mediated effects, and what plant or insect traits will dampen effects or promote cascading population changes.

ACKNOWLEDGMENTS

I want to thank my advisor, Zsofia Szendrei, for her support, guidance, and mentorship throughout my time at Michigan State. Zsofia not only gave me the tools to be an excellent scientist but demonstrated daily how to be a fantastic mentor and faculty member. Furthermore, she is willing to support not only students from her own lab, but anyone in the department who needs help. Zsofia stands up for people without power even when it requires a great investment of her own time and energy. She is always looking for opportunities to make the department and field a better place. Zsofia, I am inspired by the way you conduct yourself and will do my best to emulate these characteristics in my own career.

I would also like to thank Dr. Jared Ali for his support as a committee member as well as welcoming me to his lab as a visiting scholar for a significant portion of my degree. Jared's generosity with space, resources, and facilitation of professional connections have improved my development as a researcher. I would like to thank all my committee members—Dr. Jared Ali, Dr. Marisol Quintanilla, and Dr. Jaime Willbur—for their thoughtful comments and suggestions throughout my project development and execution. Thank you to the lab members of the Szendrei and Ali labs for their generous help and feedback: Natalie Constancio, Jen Roedel, Ray Rantz, Laura Marmolejo, Eli Bloom, Nayeli Carvajal, DeShae Dillard, Sujay Paranjape, Olivia Trase, Lizz Wagner, Nate McCartney, Alison Jennings, Anju Poudel, Faith Mihalick, and Andy Hess.

Next, I would like to extend sincere thanks to Heather Lenartson, Linda Gallagher, Erik Dams, and the rest of the business office for all their work to keep me on track with my degree requirements; for helping prepare and submit my USDA Predoctoral Fellowship application; and for facilitating my travel to many conferences, which were fantastic experiences both

professionally and personally. I also want to thank you all for your work on improving student salaries.

Thank you to all the undergraduate students who helped me on these projects: Carlisha Johnson, Simran Kaur, Ray Rantz, Faith Mihalick, and Andy Hess. Your hard work was crucial to producing these results! Thank you to all the greenhouse staff who helped me execute my experiments: Chrislyn Particka, Scott Diloreto, and Sam Gruneberg. Thank you to Monica Hufnagel for help propagating my explants. I would like to thank my funding sources: the C.S. Mott Predoctoral Fellowship, the Sustainability Scholars Program, the Michigan State Plant Science Fellowship, USDA-NIFA, and the MSU Department of Entomology.

Lastly and most importantly, thank you to my family and friends who have helped me through a series of amazing and difficult times. To my debate team—thank you for participating year after year, the student debates were a highlight of my graduate career because of you. Christina, Andrea, Josh, Elizeth, Dan, Natalie, Sujay, and all my friends at both Michigan and Penn State, spending time with you all in and out of the lab was what made grad school fun and worthwhile. Mom, Dad, and Julianna, I could not have completed this degree without your support and guidance. I love you all. Thank you!

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CHAPTER 1:

A META-ANALYSIS OF INTERACTIONS BETWEEN INSECT HERBIVORES AND PLANT-PARASITIC NEMATODES

Acknowledgment of prior publication

This chapter is a reprint of an original peer-reviewed article published in Environmental Entomology in 2022, 51(1). The original article can be found at doi: 10.1093/ee/nvab131.

ABSTRACT

Insect herbivores and plant-parasitic nematodes are global, economically devastating pests that are present in nearly every crop and natural system worldwide. Although they may be spatially separated, they indirectly interact with each other by altering both plant chemical defense and nutrition. However, the outcome of these interactions is highly variable across different focal species. We performed a meta-analysis to determine how plant and nematode traits influence insect herbivore growth and reproduction, as well as nematode abundance and reproduction. We investigated how interactions between plant-parasitic nematodes and insect herbivores influence plant biomass, carbon, and nitrogen in the roots and shoots. We found no overall effect of nematodes on insect herbivores or insect herbivores on nematodes. However, while phloem-feeding insect reproduction was not affected by nematode feeding guild or plant family, chewing insect growth increased in the presence of cyst nematodes and decreased in the presence of gall nematodes. The effect of nematodes on chewing insect herbivore growth was also affected by the focal plant family. Nematode presence did not alter plant biomass when plants were exposed to aboveground insect herbivory, but carbon and nitrogen were higher in

roots and nitrogen was higher in shoots of plants with nematodes and insects compared to plants with insects alone. Our results indicate that the mechanisms driving the outcome of aboveground-belowground interactions are still unclear, but that chewing insects may have more variable responses to nematode damage than phloem-feeders.

INTRODUCTION

Understanding the mechanisms that drive plant-mediated interactions between aboveground and belowground herbivores is increasingly important to better predict the outcomes and design strategies that can manipulate these groups for pest management (Johnson et al. 2012, Wondafrash et al. 2013, Soler et al. 2013). Belowground herbivores comprise a large and diverse set of organisms, with Coleoptera and Diptera larvae frequently the focus of these types of studies (Johnson et al. 2012). However, many plant-parasitic nematode species are economically important agricultural pests, and they also have several unique biological characteristics that set them apart from other frequently studied belowground herbivore groups. Unlike insect chewing herbivores that remove roots, two of the most damaging groups of nematodes, gall and cyst, live inside plant tissues and intimately interact with plant defenses (Mantelin et al. 2013, Li et al. 2015), forcing the plant to create a nutritional sink. While plant-parasitic nematodes and insect herbivores are both present in natural and managed ecosystems worldwide (Pimentel et al. 1991, Gatehouse 2002, van der Putten et al. 2006, Nicol et al. 2011), plant-parasitic nematodes are less likely to be recognized as an agricultural threat because their damage can be easily misidentified as disease, drought stress, or nutrient deficiency (Nicol et al. 2011); additionally, they lack an aboveground life stage that can be identified by eye, and are less apparent than insect herbivores due to their small size. However, global economic losses due

to nematode infection of crops are estimated at approximately $173 billion annually (Elling 2013).

Although plant-parasitic nematodes and foliar insect herbivores are spatially separated, they indirectly influence each other and the plant host through systemic changes to the plant's chemical defenses and nutrition (Fig. 1.1A). This plant-mediated interaction has received more attention in recent decades (Wondafrash et al. 2013, Soler et al. 2013, Heinen et al. 2018) due to its ecologically important role and potential influence on global food supply. However, the outcome of these attacks and how successfully the plant is able to defend itself is quite variable (Wondafrash et al. 2013, Soler et al. 2013), making it difficult to accurately predict the outcome of any given interaction for the plant, nematode, and insect involved. Several mechanisms may play a role in shaping the outcomes of aboveground-belowground attacks. Feeding guild is one possible mechanism since chewing and piercing-sucking/phloem-feeding insects differentially induce plant defensive pathways (Raskin 1992, Meyer et al. 1984, Walling 2000), and plant secondary metabolites are likely one of the main ways that spatially separated organisms interact (Fig. 1.1A). The jasmonic and salicylic acid pathways can play a role in defending plants against different herbivore feeding guilds (Soler et al. 2013) and are widely used herbivore defense mechanisms among plants (War et al. 2012, Ruan et al. 2019). However, these two pathways often fail to provide a complete explanation for the interactions between above- and belowground herbivores. Plant responses are known to vary to different species of herbivores from the same feeding guild (Soler et al. 2013), thus introducing a source of variability. For example, nematode interactions involving aphids have a different outcome than those involving whiteflies and leafhoppers, although all three groups are aboveground phloem-feeders (Soler et al. 2013). Plant defense strategies can rely on more specialized chemical plant defenses that are

plant family specific. For example the interaction between larval lepidopteran herbivores (*Manduca sexta* (L.) (Lepidoptera: Sphingidae) and *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae)) and root-knot nematode (*Meloidogyne incognita* (Kofoid & White) (Tylenchida: Heteroderidae)) on tobacco (*Nicotiana tabacum* (L.) (Solanales: Solanaceae)) (Kaplan et al. 2008a, 2008b). Here, nicotine is produced in the roots and transported to the shoots, which if inhibited by nematode attack, leaves the plant vulnerable to chewing herbivores (Kaplan et al. 2008b). Other compounds such as glucosinolates in cruciferous plants are also affected by and may alter the outcome of aboveground-belowground interactions (Van Dam et al. 2005, Hol et al. 2013). Understanding the general and plant-specific responses and how plant defenses are modified in the presence of both attackers will allow us to build frameworks for asking research questions and designing management strategies.

Plant-parasitic nematode biology is another factor that should be considered in our framework. Plant-parasitic nematodes, especially sedentary species like cyst and gall nematodes, have evolved an intimate relationship with plant defenses; they genetically interact with the plant to induce the formation of a syncytium or gall (Jones et al. 2013). Gall nematodes, such as *Meloidogyne* species, induce the plant to produce giant cells which then become a permanent feeding site (Jones et al. 2013), whereas cyst nematodes induce the fusion of hundreds of cells to create a syncytium (Hofmann and Grundler 2007). In both cases, the feeding site becomes a sink for photosynthetic products which the plant supports by increased metabolism (Hofmann and Grundler 2007; Fig. 1.1A). To establish the feeding site, the two nematode groups move differently through the plant: cyst nematodes move intracellularly, damaging root cells as they travel to the vascular cylinder, whereas gall nematodes migrate intercellularly and non-destructively (van Dam et al. 2018). Because these groups seem to induce different defensive

strategies within plants, the outcome of plant-mediated interactions likely differs as well (van Dam et al. 2018). The third most economically damaging group of plant-parasitic nematodes, lesion nematodes, are mobile and cause lesions leading to root cell death (Fosu-Nyarko and Jones 2016; Fig. 1.1A). Infection by lesion nematodes causes transcriptional changes in plant shoots (Zhu et al. 2014) potentially influencing aboveground herbivores. One way in which belowground feeders may change the nutritional status of plants is through the loss of root tissue causing water stress (Erb and Lu 2013) and thus changing the concentration of amino acids and sugars in phloem (Bezemer et al. 2005, Hol et al. 2013). The increased nutritional value of aboveground tissues may then lead to increased performance of foliar herbivores.

It is apparent that plant, nematode, and insect traits play a role in the outcome of plant-mediated aboveground-belowground interactions, but it is still unclear which traits act as mechanisms 1) overall, and 2) in group-specific interactions (Fig. 1.1A). We performed a meta-analysis investigating the plant-mediated effects on interactions between aboveground insect herbivores and belowground plant-feeding nematodes. We asked four main questions: 1) How does nematode presence influence insect growth and reproduction?; 2) How does plant family influence insect growth and reproduction in the presence of plant-parasitic nematodes?; 3) How does insect presence influence nematode egg production, gall and cyst production, and number of individuals? and; 4) How do plants respond to nematode-insect interactions? Answering these questions will allow us to better understand patterns of insect-nematode interactions and highlight potential avenues to manage these ecologically important relationships.

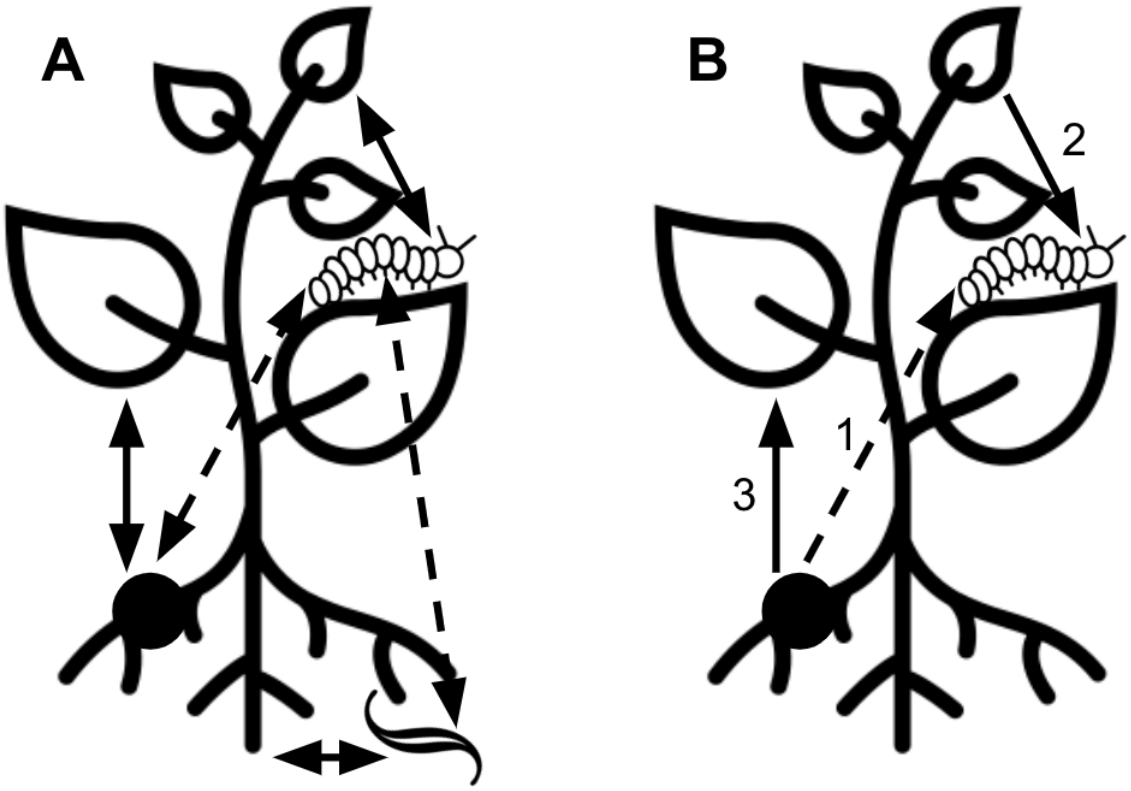

Figure 1.1. Potential interactions among plants, foliar insect herbivores and plant-parasitic nematodes (A) include direct (solid arrows) and indirect effects (dashed arrows). Results from our meta-analysis (B) confirmed that foliar chewing insect growth increased in the presence of cyst nematodes but decreased in the presence of gall nematodes (1, galls and cysts represented by closed circle); chewing insect growth was different across plant families in the presence of nematodes (2); and the presence of nematodes altered carbon and nitrogen content in plants when foliar herbivores were simultaneously feeding on plants (3).

MATERIALS & METHODS

Database construction

To design the literature search and data extraction procedures for this meta-analysis, we followed the PRISMA method (Moher et al. 2009; Fig. 1.2). We performed a 'Web of Science' search to identify the majority of papers in our database. We performed three different searches: on 28 July 2020, we searched using the terms "insect*" AND "nematode*" AND "plant-mediated" or "arthropod*" AND "nematode*" AND "plant-mediated". On 18 August 2020, we

used the search terms “arthropod*” AND “nematode*” AND “aboveground” AND “belowground”, “arthropod*” AND “nematode*” AND “aboveground” AND “belowground”, “arthropod*” AND “nematode*” AND “above ground” AND “below ground”, and “arthropod*” AND “nematode*” AND [“aboveground” OR “above-ground” OR “above ground”] AND “belowground”. Finally, on 5 October 2020 we used the search terms arthropod* AND nematode* AND plant feeding. In total, we identified 506 papers from the ‘Web of Science’ search. We also searched the literature cited sections of two recent reviews (Wondafrash et al. 2013, Heinen et al. 2018) to identify additional papers, and found 5 that had not been previously included.

For inclusion in the database, all papers had to have at least one species of insect herbivore feeding aboveground and one plant-feeding nematode belowground. We screened titles and abstracts of all papers identified in our search and excluded 448 that did not fit our criteria. From the remaining studies, 63 full papers were assessed, and 37 total were included in the database representing 75 insect-nematode responses and 156 plant responses (Table S1.1). Papers included in the database met the following criteria: 1) included mean and a measure of variance (standard deviation or standard error of the mean), 2) reported number of replicates, 3) included a nematode- or insect-free control, 4) did not utilize artificial damage, 5) insect and nematode herbivory occurred on the same plant, and 6) paper was available in English. Twenty-six were excluded from 63 assessed papers because they did not meet one or more of the criteria listed above (Table S1.2). Out of the remaining 37 papers, 25 were used in the quantitative analyses, and the rest were summarized. We did not include review articles.

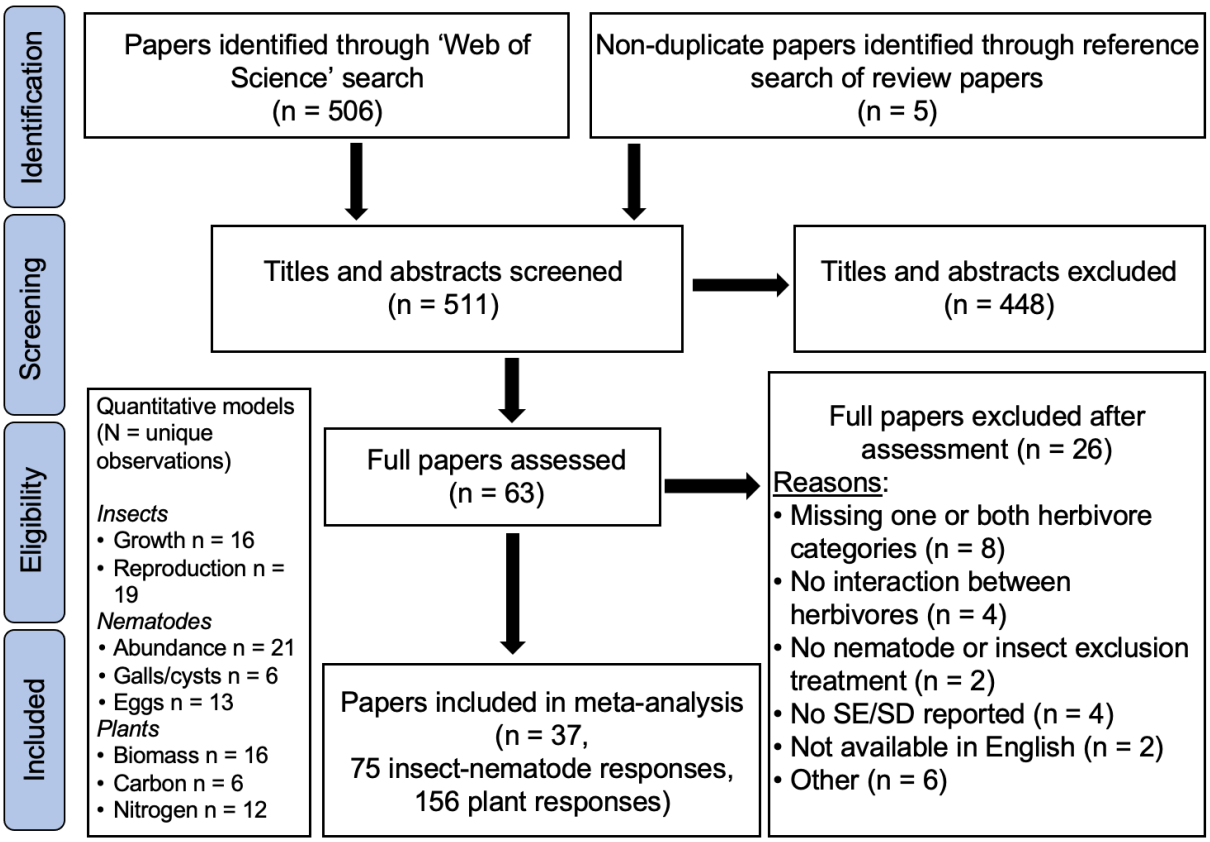

Figure 1.2. PRISMA workflow: published studies for the meta-analysis were located by searching 'Web of Science' and the references provided in two review articles. Full papers included are in Table S1.2 and those excluded are in Table S1.3.

Insect and nematode response

Data extraction

We collected basic information from the 37 papers in our database including year published, author, title, journal, study type (conducted in the laboratory or field), and family and species for the focal plants, nematodes, and insects, including any natural enemies. Nematode and insect feeding guild was recorded as it was reported in the paper or determined by reading existing literature on the species. We recorded information about the type of herbivore response: reproduction, growth, and behavior. For all response variables we included in our database the mean, standard deviation or standard error, number of control and treatment replicates, and

number of insects and nematodes per treatment. If a study reported more than one response variable or the same response variable for more than one focal species, we included all potential variables and species as separate entries in the database. When replicate number was reported as a range (e.g. 7-8), we used the lower value in our analyses. If data was taken over time for the same study, we chose the date closest to the values reported by other studies (typically one week post initiation of the experiment).

Plant response

Data extraction

From the 156 plant responses identified in our database (Fig. 1.2), only plant family (n = 34, 14 papers qualified to be included in quantitative analysis; all papers reported plant family), biomass (n = 19, 6 papers), carbon (n = 6, 2 papers), and nitrogen (n = 12, 5 papers) responses occurred frequently enough to be included in the meta-analysis. Those response variables were analyzed in the same way as the insect responses, and we followed all the procedures outlined above. Plant responses included plant defense, growth, nutrition, reproduction, water content, and amino acids.

For plant responses that did not occur frequently enough to be used in a meta-analysis, we grouped responses by category (plant defense compounds, carbon/nitrogen (stable isotopes or C/N ratio), amino acids, or other; Table S1.3). We recorded the plant families, number of studies, references, and trend. For the trend, we used a '↑'symbol if the treatment response was greater than the control, a '↓' symbol if the treatment response was less than the control, and shaded the symbol if the difference was statistically significant in the original reference.

Statistical Analyses

All statistical analyses were conducted in R (Bates et al. 2015, R Core Team 2017), using the *metafor* package (Viechtbauer 2010) to create mixed-effects models. We calculated Hedge's g, the standardized mean difference, as our effect size since Hedge's g is more robust with small sample sizes (Hedges and Olkin 1985). A positive effect size means that the treatment response was greater than the control response; for example, if the response variable is insect growth, insect herbivores weighed more in the presence of nematodes than in the control nematode-free treatment. A negative effect size means that the treatment response was smaller than the control response. An effect size of 0.8 or greater is considered large, 0.5-0.8 is considered moderate, and 0.2 or smaller is considered small (Cohen 1988). Our effect sizes were considered to be significant if the 95% confidence interval did not overlap zero. We used 'Title' as a random effect for all models to control for different experiments reported in the same study. For models where fixed effects were significant, we report z-values and p-values for the fixed effects. For models where fixed effects were not significant, we report Q_m values, an omnibus test of moderators against the null hypothesis that the true value of all coefficients is equal to zero (Viechtbauer 2010).

We tested for publication bias using funnel plots (Fig. S1.1) and Egger's test (Egger et al. 1997) with the 'regtest' function in the *metafor* package, as well as forest plots (Fig. S1.2) and quantile-quantile plots (Fig. S1.3). We used models without the random effects to test for publication bias because 'regtest' will not accept random effects. For response variables insect growth and reproduction, nematode egg production and total individuals, and plant nitrogen, Egger's test yielded a nonsignificant p-value ($p > 0.05$), indicating that publication bias was not detected. However, Egger's test returned a significant p-value ($p < 0.05$) for plant carbon and

nematode gall and cyst production, which also had a non-linear quantile-quantile plots (Fig. S1.3). This is likely due to the small sample size of our carbon response variable (n = 6) and gall/cyst response variable (n = 6). Therefore, these results should be interpreted with caution.

RESULTS

Of the studies in our database, 6 experiments occurred in the field and 43 in the lab, many of which included multiple combinations of plant, insect, and nematode families (Table 1.1). A combination was counted as unique if it occurred in a unique experiment or combined different families, species, or varieties of insects, plants, and nematodes. These studies spanned seven unique plant families, 13 insect families, and four nematode families (Table 1.1). Often, certain families were used more frequently within a category: solanaceous plants were used in 27 data points, whereas the next most frequently used plant family, Brassicaceae, occurred 15 times (Table 1.1). Although the number of insect families was greater than plants and nematodes, the majority of studies that used insects were from Aphididae: 35 occurrences, while the next most common family, Noctuidae, were used in 13 experiments (Table 1.1). Focal nematodes were mostly represented by species in Heteroderidae (49 data points), followed by studies that looked at nematode communities (15 data points; Table 1.1).

Plant families were unequally used in experiments with certain insect families. Some were relatively evenly distributed between chewers and phloem feeders: for example, plants in the family Fabaceae were used in five experiments with Aphididae (phloem-feeder), five with Noctuidae (chewing), and one time with an insect community (Table 1.1). Others were usually used in experiments with insects from a single feeding guild: 87% of experiments with brassicaceous plants used Aphididae (Table 1.1). This uneven distribution is true for many

family pairings: noctuid insects were only evaluated with nematodes from one family (Heteroderidae) (Table 1.1).

There was also a wide spread in plant responses to nematode-insect interactions. Many of these occurred infrequently so they could not be used in the meta-analysis (Table S1.3). These fell into five categories: plant defense compounds (23 response variables), carbon/nitrogen (3 response variables distinct from the carbon and nitrogen values used in the meta-analysis), amino acids (2 response variables), and 'other' (6 response variables). Some of the plant defense compounds, such as nicotine, are compounds that are specific to a particular plant family. However, even some of the most universal compounds were not frequently tested: jasmonic acid and salicylic acid were each only measured in two studies (Table S1.3). Therefore, chemical defense responses were not analyzed quantitatively.

How does nematode presence influence insect growth and reproduction?

Overall, there was no effect of nematode presence on chewing insect growth ($z = -1.55$, $p = 0.2$, $N = 16$, unique papers = 4) or phloem-feeding insect reproduction ($z = 0.749$, $p = 0.45$, $N = 19$, unique papers = 11). When nematodes were separately analyzed by feeding guild (gall, cyst, lesion), gall forming nematodes, which move intercellularly, reduced growth of chewing insects ($z = -3.70$, $p = 0.0002$, Fig. 1.3A). Cyst- forming nematodes increased growth ($z = 3.10$, $p = 0.002$). Lesion nematodes were not included in the growth analysis because they were only represented in one unique data point. Reproduction of phloem-feeding insects was not affected by nematode feeding guild ($Q_M = 4.29$, $df = 3$, $p = 0.23$, Fig. 1.3B).

How does plant family influence insect growth and reproduction?

Chewing insect growth increased on Fabaceae ($z = 3.52$, $p = 0.0004$) but decreased on Amaranthaceae ($z = -3.35$, $p = 0.0008$) and Solanaceae ($z = -2.91$, $p = 0.004$) when nematodes

were present (Fig. 1.3C). Phloem-feeding insect reproduction in the presence of nematodes was not affected by plant family ($Q_M = 1.29$, df = 3, p = 0.73, Fig. 1.3D).

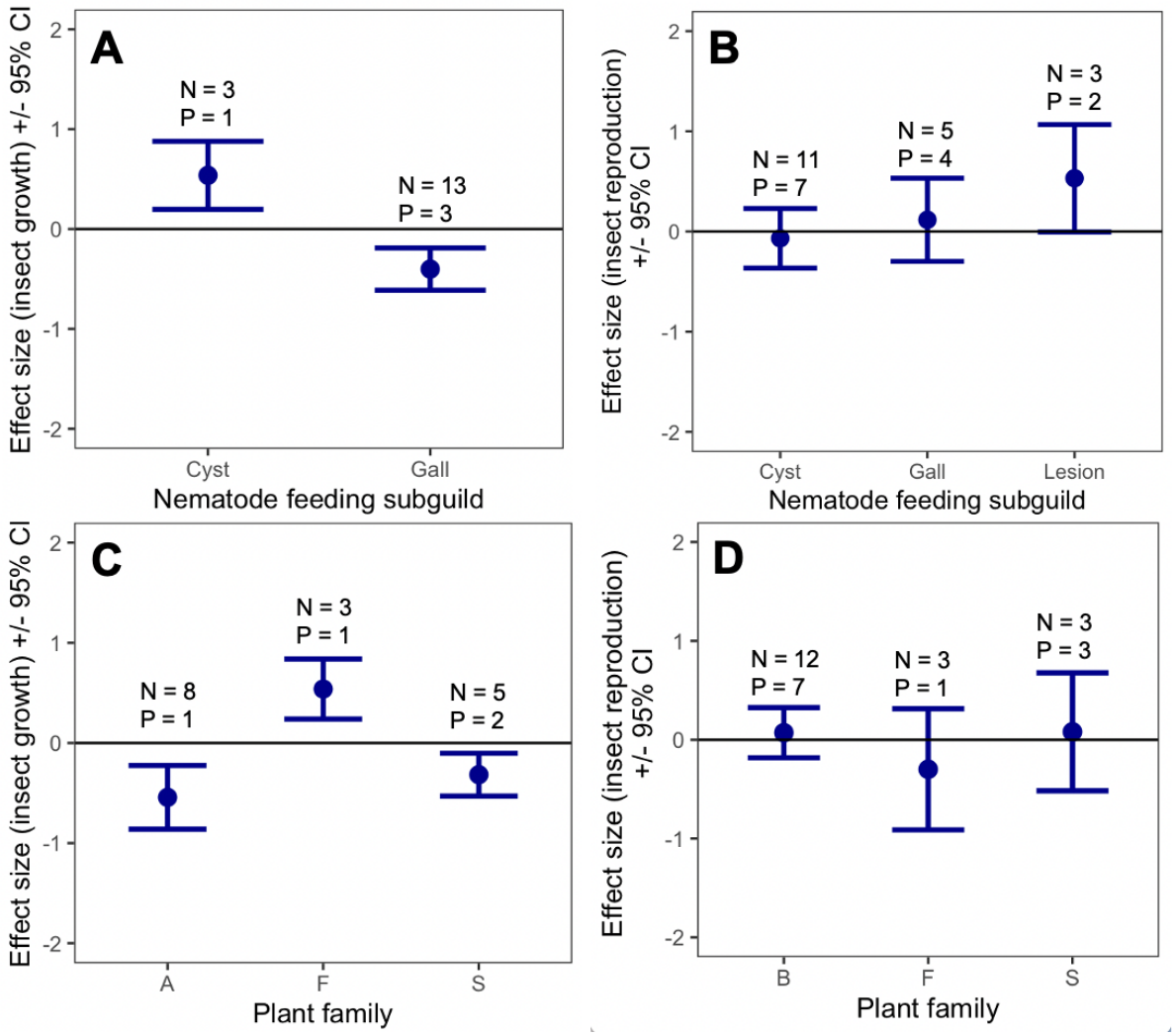

Figure 1.3. Effect of nematode feeding guild on chewing insect growth (A) and phloem-feeding insect reproduction (B); effect of plant family on chewing insect growth (C) and phloem-feeding insect reproduction (D) in the presence of nematodes (A = Amaranthaceae, B = Brassicaceae, F = Fabaceae, S = Solanaceae). The effect size compares the response variable on plants with nematode and insect herbivores (treatment plants) to the response variable on plants with insect herbivores only (control plants). Response variable is effect size (Hedge's g, standardized mean difference) +/- 95% confidence interval (CI). N = number of unique observations included; P = number of unique papers observations were drawn from.

How does insect presence influence nematode egg production, gall and cyst production, and number of individuals?

Overall, there was no effect of insect presence on nematode egg production (z = 0.57, p = 0.56, N = 13, unique papers = 4), gall and cyst production (z = 0.94, p = 0.35, N = 6, unique papers = 3), or number of individual nematodes in roots or soil (z = 1.34, p = 0.18, N = 21, unique papers = 7). Nematode feeding guild and insect feeding guild (chewing vs. phloem-feeding) had no effect on egg production (Q_M = 3.18, df = 2, p = 0.2, Fig. S4A and Q_M = 1.87, df = 2, p = 0.39, Fig. S4B respectively) or gall and cyst production (Q_M = 0.64, df = 2, p = 0.73, Fig. S1.5A and Q_M = 0.49, df = 2, p = 0.78, Fig. S1.5B respectively). The number of cyst nematodes increased in the presence of insects (z = 1.97, p = 0.05, Fig. S1.6A) while plant-feeding nematode communities in the soil were unaffected (z = -0.29, p = 0.78, Fig. S1.6A); insect feeding guild did not influence overall nematode abundance in roots or soil (Q_M = 2.41, df = 2, p = 0.3, Fig. S1.6B).

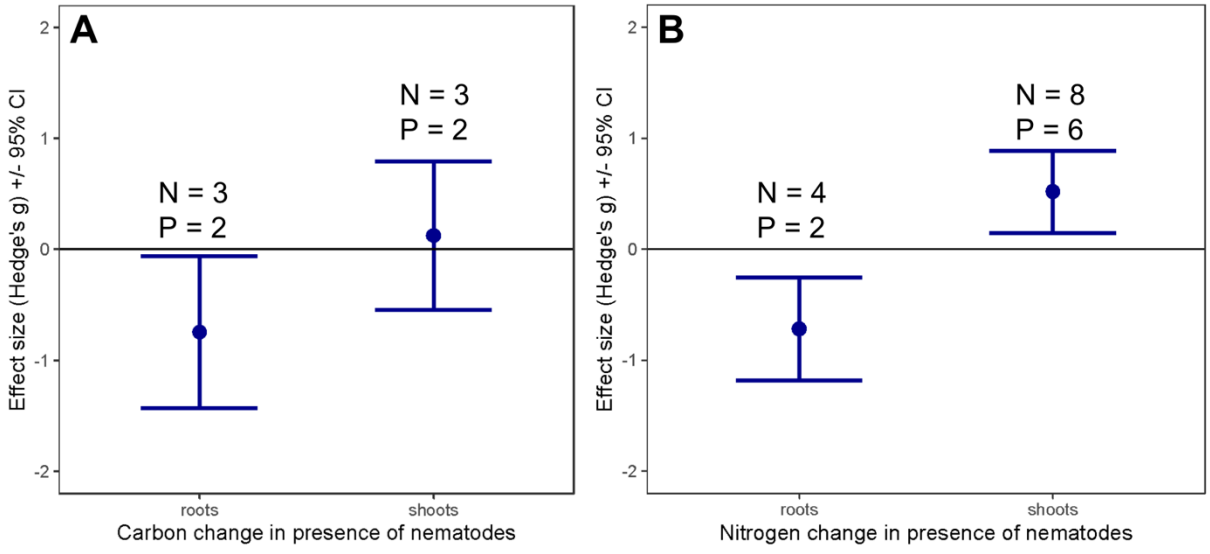

Figure 1.4. Effect of insect feeding on change in A) carbon and B) nitrogen in roots and shoots in nematode treatments compared to control treatments. Response variable is effect size

Figure 1.4 (cont'd)
(Hedge's g, standardized mean difference) +/- 95% confidence interval (CI). N = number of unique observations included; P = number of unique papers observations are drawn from.

How do plants respond to nematode-insect interactions?

Nematode presence did not alter plant biomass change in response to insects in roots or shoots ($Q_M = 0.27$, df = 2, p = 0.87), and plant biomass change in the presence of nematodes did not change based on insect feeding guild ($Q_M = 0.83$, df = 2, p = 0.66). However, the amount of carbon and nitrogen in roots and shoots was altered by nematode presence ($Q_M = 9.70$, df = 2, p = 0.008 and $Q_M = 23.76$, df = 2, $p < 0.0001$, respectively). Plants with nematodes had lower carbon in roots (z = -2.13, p = 0.03, Fig. 1.4A), but carbon in the shoots was unchanged (z = 0.36, p = 0.72). Nitrogen was lower in the roots (z = -3.04, p = 0.002) and higher in shoots (z = 2.73, p = 0.006) in treatments with nematodes compared to controls (Fig. 1.4B).

DISCUSSION

A growing body of literature investigating plant-mediated insect-nematode interactions has established that insects and nematodes affect each other in a variety of ways, potentially through mechanisms such as induction of plant defenses or nutritional changes in plant tissues (Soler et al. 2012, Wondafrash et al. 2013). Herbivore feeding mode is one of the key determinants for inducing species-specific plant defenses and this seems to be supported by our meta-analysis (Soler et al. 2013, van Dam et al. 2018). While our meta-analysis indicated a lack of overall nematode effect on insect performance, analyzing nematode feeding modalities separately revealed differences across these groups. Reviews of insects and plant pathogenic nematodes

Table 1.1. Plant, insect, and nematode families and associated experiments from studies in the meta-analysis database. Columns 2 and 4 (laboratory, field) denote the location of the studies. References are listed in Table S1.1.

	Family	No. unique combin-ations	Lab	Field	Plant families	Insect families	Nematode families	References
Plants	Amaranthaceae	3	3	0	NA	Aphididae (1), Chrysomelidae (2)	Heteroderidae (3)	Hol et al. 2010, Wei et al. 2016
	Brassicaceae	15	15	0		Aphididae (13), Pieridae (1), Tetranychidae (1)	Heteroderidae (10), Pratylenchidae (3), NA (2)	Hol et al. 2013, Hol et al. 2016, Kabouw et al. 2011, Kammerhofer et al. 2015, Kutyniok and Muller 2012, Kutyniok and Muller 2013, Kutyniok et al. 2014, van Dam et al. 2005, van Dam et al. 2018
	Fabaceae	11	9	2		Aphididae (5), Noctuidae (5), NA (1)	Heteroderidae (11)	Alston et al. 1991, Hong and Gratton 2010, Li et al. 2017, McCarville et al. 2014, Russin et al. 1993, Vockenhuber et al. 2013
	Malvaceae	1	1	0		Noctuidae (1)	Heteroderidae (1)	Olson et al. 2008
	Plantaginaceae	1	1	0		Aphididae (1)	Pratylenchidae (1)	Wurst and van der Putten 2007
	Poaceae	13	11	2		Aphididae (4), Crambidae (1), Delphacidae (4), Erebidae (2), Romalediae (2)	Heteroderidae (1), NA (12)	Bezemer et al. 2005, Huang et al. 2012, Fu et al. 2001, Tiwari et al. 2009, Vandegehuchte et al. 2010, Vestergard et al. 2004, Zhou et al. 2017
	Solanaceae	27	20	7		Aleyrodidae (1), Aphididae (11), Gelechiidae (1), Noctuidae (7),	Sphingidae (6), NA (1) Heteroderidae (23), Helicotylenchus (2),	Arce et al. 2017, Guo and Ge 2017, Hoysted at al. 2017, Kafle et al. 2017, Kaplan et al. 2008a, Kaplan et al. 2009, Kaplan et al. 2011, Li et al. 2020,

Table 1.1 (cont'd)

						Tylenchorhynchus (2)	Schoning and Wurst 2016
	NA (Community)	1	0	1	Acrididae (1)	NA (1)	De Deyn et al. 2007
Insects	Acrididae	1	0	1	NA (1)	NA (1)	De Deyn et al. 2007
	Aleyrodidae	1	1	0	Solanaceae (1)	Heteroderidae (1)	Guo and Ge 2017
	Aphididae	35	32	3	Amaranthaceae (1), Brassicaceae (13), Fabaceae (5), Plantaginaceae (1), Poaceae (4), Solanaceae (11)	Helicotylenchus (1), Heteroderidae (24), Pratylenchidae (3), Tylenchorhynchus (1), NA (6)	Bezemer et al. 2005, Hol et al. 2010, Hol et al. 2013, Hol et al. 2016, Hong and Gratton et al. 2010, Hoysted et al. 2017, Kabouw et al. 2011, Kafle et al. 2017, Kaplan et al. 2009, Kaplan et al. 2011, Kutyniok and Muller 2012, Kutyniok and Muller 2013, Kutyniok et al. 2014, Li et al. 2020, McCarville et al. 2014, van Dam et al. 2018, Vandegehuchte et al. 2010, Vestergard et al. 2004, Wurst and van der Putten 2007
	Chrysomelidae	2	2	0	Amaranthaceae (2)	Heteroderidae (2)	Wei et al. 2016
	Crambidae	1	1	0	Poaceae (1)	Heteroderidae (1)	Tiwari et al. 2009
	Delphacidae	4	4	0	Poaceae (4)	NA (4)	Huang et al. 2012
	Erebidae	2	2	0	Poaceae (2)	NA (2)	Zhou et al. 2017
	Gelechiidae	1	1	0	Solanaceae (1)	Heteroderidae (1)	Arce et al. 2017
	Noctuidae	13	11	2	Fabaceae (5), Malvaceae (1), Solanaceae (7)	Heteroderidae (13)	Alston et al. 1991, Kafle et al. 2017, Kaplan et al. 2008a, Kaplan et al. 2009, Li et al. 2017, Olson et al. 2008, Russin et al. 1993
	Pieridae	1	1	0	Brassicaceae (1)	Pratylenchidae (1)	Van Dam et al. 2005
	Romaleidae	2	0	2	Poaceae (2)	NA (2)	Fu et al. 2001
	Sphingidae	6	4	2	Solanaceae (6)	Helicotylenchus (1), Heteroderidae (4),	Kaplan et al. 2008a, Kaplan et al. 2009, Schoning and Wurst 2016

Table 1.1 (cont'd)

							Tylenchorhynchus (1)	
	Tetranychidae	1	1	0	Brassicaceae (1)		Heteroderidae (1)	Kammerhofer et al. 2015
	NA (Community)	2	0	2	Fabaceae (1), Solanaceae (1)		Heteroderidae (2)	Kaplan et al. 2009, Vockenhuber et al. 2015
Nematodes	Helicotylenchus	2	0	2	Solanaceae (2)	Aphididae (1), Sphingidae (1)	NA	Kaplan et al. 2009
	Heteroderidae	49	44	5	Amaranthaceae (3), Brassicaceae (10), Fabaceae (11), Malvaceae (1), Poaceae (1), Solanaceae (23)	Aleyrodidae (1), Aphididae (24), Chrysomelidae (2), Crambidae (1), Gelechiidae (1), Noctuidae (13), Sphingidae (4), Tetranychidae (1), NA (2)		Alston et al. 1991, Arce et al. 2017, Guo and Ge 2017, Hol et al. 2010, Hol et al. 2013, Hol et al. 2016, Hong and Gratton 2010, Hoysted et al. 2017, Kafle et al. 2017, Kammerhofer et al. 2015, Kaplan et al. 2008a, Kaplan et al. 2009, Kaplan et al. 2011, Kutyniok and Muller 2012, Kutyniok and Muller 2013, Kutyniok et al. 2014, Li et al. 2017, Li et al. 2020, McCarville et al. 2014, Olson et al. 2008, Russin et al. 1993, Schoning and Wurst 2016, Tiwari et al. 2009, van Dam et al. 2018, Vockenhuber et al. 2013, Wei et al. 2016
	Pratylenchidae	4	4	0	Brassicaceae (3), Plantaginaceae (1)	Aphididae (3), Pieridae (1)		Hol et al. 2016, van Dam et al. 2005, Wurst and van der Putten 2007
	Tylenchorhynchus	2	0	2	Solanaceae (2)	Aphididae (1), Sphingidae (1)		Kaplan et al. 2009

Table 1.1 (cont'd)

	NA (Community)	15	12	3	Brassicaceae (2), Poaceae (12), NA (1)	Acrididae (1), Aphididae (6), Delphacidae (4), Erebidae (2), Romaleidae (2)	Bezemer et al. 2005, De Deyn et al. 2007, Fu et al. 2001, Huang et al. 2012, Kabouw et al. 2011, Vandegehuchte et al. 2010, Vestergard et al. 2004, Zhou et al. 2017

(Wondafrash et al. 2013) and insects and other belowground herbivores (Soler et al. 2012, 2013, Johnson et al. 2012) reported similar variability for positive, neutral, or negative impacts of nematodes on aboveground insects. The three groups of nematodes in our study represent different ways of damaging the plant, migration strategies, and feeding structures, thereby causing plants to be induced in different ways. Nematodes can manipulate plant hormonal signaling and suppress plant defenses in above ground plant tissues (Hamamouch et al 2011), thus it is expected that they will have different impacts on aboveground herbivores. For example, cyst and root-knot nematodes affected aphid preference and performance in opposite ways and this was due to systemically induced responses by the nematodes (van Dam et al. 2018). The dataset available to us was limited (for example, combinations of cyst nematodes with chewing insects; Fig. 1.3), but they allow us to build models we discuss below for mechanisms that might be playing a significant role in driving these interactions.

Reciprocal effects of nematodes and aboveground herbivores

Understanding the effect of plant-parasitic nematodes on aboveground herbivores is crucial for managing their populations in agriculture. Plant-mediated effects of nematodes on insect growth and reproduction were the most common ways for studies in the meta-analysis to report the outcomes of these interactions, and therefore the data we used for the quantitative analyses. This makes it challenging to compare side-by-side the effect of nematodes on these two aboveground herbivore groups. Collecting additional data such as body mass change over time for phloem-feeders, reproduction of chewing insects, feeding time/frequency, and distribution on the plant could allow for more direct comparisons between feeding guilds. However, these can sometimes be more challenging or time-consuming to measure, hence the lack of data in the literature.

While the overall outcome of nematodes on insect herbivores was neutral, in the case of chewing herbivores, nematodes influenced insects in opposite ways based on their feeding mode (Fig. 1.3A). Interestingly, cyst nematodes increased insect growth (Fig. 1.1B); this is somewhat counterintuitive since these types of nematodes cause cell damage and death as they feed (Fosu-Nyarko and Jones 2016, van Dam et al. 2018), similarly to the damage done by chewing herbivores. As a result, we would have expected to see that the nematode and chewing insect damage may be inducing similar defense pathways leading to a stronger plant defense response compared to when a single damager is attacking the plant (Kaplan et al. 2008b). Gall nematodes migrate non-destructively (van Dam et al. 2018) and would not be expected to induce the same defense mechanisms. Gall and cyst nematodes also respond differently to the phytohormone ethylene, which is involved in both plant defense (Ecker and Davis 1987) and growth (Burg 1973). The ethylene pathway promotes resistance to root-knot (gall) nematodes, but plants with ethylene in the roots are susceptible to cyst nematodes (reviewed in Mantelin et al. 2013, Li et al. 2015). This also supports our result that overall, insects did not influence nematode performance, but insect presence (which induces ethylene) led to a higher abundance of cyst nematodes. This damage above- and belowground could then influence species-specific colonization patterns of other herbivores. Since nematode feeding can change plants' primary compound composition (Hofmann et al. 2010), this may lead to increased growth in chewing insects. Some specialist herbivores may also be able to counter nematode-induced plant defenses through sequestration or other mechanisms which allow them to overcome the negative effects of secondary plant chemicals.

Positive effects of belowground herbivores on phloem-feeders are assumed to be mediated by an enhancement of nutritional quality, but this may only be temporary (Soler et al

2013). Gall and lesion nematodes had a non-significant positive trend towards increased reproduction in our meta-analysis, but cyst nematodes showed a non-significant negative trend. The arrival sequence onto plants and the time spent feeding on plants can drastically change the outcomes of these interactions (Erb et al. 2011, Johnson et al. 2012). Our meta-analysis did not investigate the directionality and sequence of interactions and it is likely that there is considerable variability in effects based on the species involved and their arrival sequence. A previous meta-analysis found that when belowground or aboveground herbivores were introduced first, there were no significant effects on performance, only when these herbivores were simultaneously introduced (Johnson et al. 2012). Out of all 25 papers included in quantitative analyses that investigated insect or nematode responses, only four introduced herbivores simultaneously, indicating a key gap in the literature. All the interactions in our chewing insect growth dataset introduced nematodes first; only one interaction measuring phloem-feeders' reproduction was simultaneous. Jasmonic acid, typically a response to chewing herbivores and cell damage, can induce resistance to both chewing herbivores and phloem-feeders (Smith and Boyko 2007, Kuśnierczyk et al. 2011), whereas salicylic acid typically induces resistance to phloem-feeders only and can interfere with jasmonic acid signaling (Caarls et al. 2015). In fact, some studies suggest that phloem-feeders may induce salicylic acid specifically to suppress the jasmonic acid pathway and improve their performance (Zarate et al. 2007, Zhang et al. 2013). This could explain why there was no difference in the reproduction of phloem-feeders, but there was a difference in growth of chewers: any nematode attack may induce pathways in the plant to defend against aphids, but only cyst and lesion nematodes would induce plant defenses that are effective against chewing insects. However, to confirm this hypothesis, a larger pool of studies, especially with lesion nematodes, will need to be examined.

Plant response to nematode-insect interactions

Two main mechanisms are typically considered for plant responses to spatially isolated herbivores: chemical defense and changes in nutrition. The studies we examined reported a wide range of chemical defense response variables, many of them family specific, with mixed responses (Table S1.3). Highly conserved and systemic responses such as jasmonic acid, salicylic acid, and ethylene are often considered potential chemical defense mechanisms (Soler et al. 2013). However, these pathways appear to be eliciting different chemical responses to nematodes in different plant families, which have varying effects on insect herbivores. If jasmonic acid and salicylic acid were inducing similar pathways in the majority of plant-mediated outcomes, we would expect to see similar responses across plant family since both jasmonic acid and salicylic acid are broadly utilized, conserved defenses (Raskin 1992, Meyer et al. 1984, Walling 2000). This was however not the case: chewing herbivore growth outcomes differed based on plant family, but phloem-feeding insect reproduction was similar (Fig. 1.1B). One potential mechanism driving this difference is that chewers are more susceptible to secondary metabolites than phloem-feeders, since phytotoxins are typically stored in cells (Larsson 1989, Soler et al. 2012).

The second mechanism, nutrition, seems more likely to be a widespread driver of aboveground-belowground interactions. We found that nematode presence reduced carbon and nitrogen in the roots of plants and increased nitrogen in plant shoots. This supports the ‘Stress Response Hypothesis’ (Masters et al. 1993), which states that water stress caused by feeding damage may lead to higher levels of soluble nitrogen and carbon in plant foliage. Nitrogen is often a limiting factor of insect growth and reproduction (Nevo and Coll 2001, Bala et al. 2018), so increased amounts of these compounds in plant tissues could promote herbivore performance

(Masters et al. 1993). However, experiments where plants are treated with synthetic fertilizers indicate that there are species-specific differences in insects' ability to use increased nitrogen in the plant (Emden 1966). This could be the reason for a lack of an overall positive effect of nematode presence on phloem-feeders. Changes in primary metabolites can shape aboveground outcomes when belowground non-nematode herbivores, such as wireworms or root-aphids, are damaging the plant (Gange and Brown 1989, Johnson et al. 2009, 2013). Specifically, root chewers often have a pronounced negative effect on root biomass (Zvereva and Kozlov 2012) compared to nematodes, which did not have an overall effect on root biomass in our meta-analysis. This difference might explain why the results were stronger with belowground chewing organisms.

Influence of insect-nematode interactions on natural enemies

Nematode damage also has the potential to strongly influence natural enemies, a topic which is recently gaining more attention, although there were still too few papers in our dataset to include it in our quantitative analysis. Parasitoids are known to respond differently to volatile blends from plants with or without nematode damage belowground, typically because blends from plants with belowground damage contain more insect repellents (Dicke et al. 2009, Soler et al. 2012). Importantly, this response is also altered by whether nematode-damaged plants are offered in a mixed or clumped distribution (Soler et al. 2012). The three studies in our dataset that used parasitoid wasps and results were mixed, with no effect of nematode presence on foraging choice for *M. croceipes* (Cresson) (Hymenoptera: Braconidae) (Olson et al. 2008), a positive effect on survival for *A. colemani* (Viereck) (Hymenoptera: Braconidae) (Bezemer et al. 2005), and a negative effect on size and fecundity of *M. pulchricornis* (Wesmael) (Hymenoptera: Braconidae) (Li et al. 2017). However, parasitoids are constrained by the performance of their

hosts (Li et al. 2017), so if hosts are negatively affected by nematode presence, parasitoid performance will likely be reduced as well, regardless of host choice. Insect predator response to nematode-infested plants has been largely unexplored, although predators are known to be attracted to herbivore-induced volatile cues (reviewed in Hare 2011) and volatile cues can be altered by plant feeding nematodes (Olson et al. 2008). Additionally, predators are not constrained by the performance of a single host, which means that they may outperform parasitoids if insect herbivores are negatively influenced by nematode presence.

CONCLUSIONS

Our results support previous views that above- and belowground herbivores interact via the plant host, which responds to different traits of these organisms (e.g. feeding guild) using both generic responses, such as plant hormones or primary metabolites, and specialized responses like secondary metabolites used for plant defense. Nematode presence increases nitrogen availability in plant shoots, but this does not consistently improve outcomes for insects. This is true even for phloem-feeders, which are hypothesized to be more strongly influenced by root herbivory. Instead, it appears that family-specific secondary metabolites, perhaps in combination with systemic, induced responses, have a more significant role in mediating outcomes. However, these dynamic systems are challenging to study and consistent, quantitative measurements in a variety of study systems are still a limiting factor in understanding plant-parasitic nematode-insect herbivore interactions. Future studies with more plant families, insect and nematode species will help strengthen our understanding of mechanisms that are broadly applicable and those that are family-specific to predict the outcomes of their interaction.

ACKNOWLEDGEMENTS

We thank E. Davidson-Lowe, J. Ali, J. Willbur, M. Quintanilla, E. Bloom, J. Zavalnitskaya, and

N. Constancio for thoughtful comments and feedback. We thank A. Buchanan for providing statistical support and advice.

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APPENDIX 1

Table S1.1. Papers included in the meta-analysis.

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Table S1.2. We assessed 63 full papers for the meta-analysis and excluded the following 26 based on the criteria listed in the methods.

Year	Author	Title	Journal	Reason for exclusion	Notes
2004	Bezemer et al.	Above- and belowground trophic interactions on creeping thistle (*Cirsium arvense*) in high- and low-diversity plant communities: potential for biotic resistance?	Plant Biology	No SD/SE reported	
2010	Bonte et al.	Local adaptation of aboveground herbivores towards plant phenotypes induced by soil biota	PLoS One	Missing one or both herbivore categories	No aboveground insect herbivore
2013	De Roissart et al.	The presence of root-feeding nematodes--Not AMF--Affects an herbivore dispersal strategy	Acta Oecologia	Other	Only reported results for average mite density
2016	Filgueiras et al.	Eliciting maize defense pathways aboveground attracts belowground biocontrol agents	Scientific Reports	Missing one or both herbivore categories	No plant-feeding nematode
2020	Grunseich et al.	Risky roots and careful herbivores: sustained herbivory by a root-feeding herbivore attenuates indirect plant defences	Functional Ecology	Missing one or both herbivore categories	No plant-feeding nematode
2012	Heeren et al.	The interaction of soybean aphids and soybean cyst nematodes on selected resistant and susceptible soybean lines	Journal of Applied Nematology	Not available in English	
2011	Hong et al.	Soybean aphid and soybean cyst nematode interactions in the field and effects on soybean yield	Journal of Economic Entomology	No SD/SE reported	

Table S1.2 (cont'd)

2018	Hoysted et al.	Aphid colonization affects potato root exudate composition and the hatching of a soil borne pathogen	Frontiers in Plant Science	Missing one or both herbivore categories	No herbivores
2015	Huang et al.	Effects of intraspecific variation in rice resistance to aboveground herbivore, brown planthopper, and rice root nematodes on plant yield, labile pools of plant, and rhizosphere soil	Biology and Fertility of Soils	No nematode or insect exclusion treatment	
2008	Kaplan et al.	Effects of plant vascular architecture on aboveground-belowground-induced responses to foliar and root herbivores on *Nicotiana tabacum*	Journal of Chemical Ecology	No nematode or insect exclusion treatment	
2015	Kostenko et al.	Plant diversity and identity effects on predatory nematodes and their prey	Ecology and Evolution	No interaction between aboveground-belowground herbivores	
2014	Kutyniok et al.	Local and systemic transcriptional responses to crosstalk between above- and belowground herbivores in Arabidopsis thaliana	Plant Signaling and Behavior	Other	Evolution experiment
2017	Machado et al.	Aboveground herbivory induced jasmonates disproportionately reduce plant reproductive potential by facilitating root nematode infestation	Plant, Cell & Environment	Missing one or both herbivore categories	No aboveground insect herbivore
2018	Moreira et al.	Interactions between plant defence signalling pathways: Evidence from bioassays with insect herbivores and plant pathogens	Journal of Ecology	Missing one or both herbivore categories	No aboveground insect herbivore

Table S1.2 (cont'd)

2017	Mundim et al.	A whole-plant perspective reveals unexpected impacts of above- and belowground herbivores on plant growth and defense	Ecology	Missing one or both herbivore categories	No plant-feeding nematode
2020	Musedeli et al.	Additive interaction between a root-knot nematode *Meloidogyne javanica* and a root-feeding flea beetle *Longitarsus bethae* on their host Lantana camara	Pest Management Science	No interaction between aboveground-belowground herbivores	
2009	Ramirez and Snyder	Scared sick? Predator-pathogen facilitation enhances exploitation of a shared resource	Ecology	Missing one or both herbivore categories	No plant-feeding nematode
2014	Ramirez and Spears	Stem nematode counteracts plant resistance of aphids in alfalfa, *Medicago sativa*	Journal of Chemical Ecology	No interaction between aboveground-belowground herbivores	
2018	Rusman et al.	Dealing with mutualists and antagonists: Specificity of plant-mediated interactions between herbivores and flower visitors, and consequences for plant fitness	Functional Ecology	Missing one or both herbivore categories	No plant-feeding nematode
2017	Som et al.	Dynamics of belowground volatile diffusion and degradation	Rhizosphere	No interaction between aboveground-belowground herbivores	
2005	Van Ruijven et al.	Interactions between spatially separated herbivores indirectly alter plant diversity	Ecology Letters	Missing one or both herbivore categories	No belowground nematode
2004	Wardle et al.	Linking aboveground and belowground communities: the indirect influence of aphid species identity and diversity on a three trophic level soil food web	Oikos	Other	Mechanical damage

Table S1.2 (cont'd)

2005	Wardle et al.	Trickle-down effects of aboveground trophic cascades on the soil food web	Oikos	Other	Meta-analysis
2019	Xiang et al.	Comparison of effects of root-knot nematode on the growth and nutrient utilization of two herbivores with different diet breadths	Journal of Environmental Entomology	Other	Nematode treatments included whole community without reporting results for plant-feeders specifically
2016	Zhou et al.	The fungal endophyte *Chaetomium globosum* negatively affects both above- and belowground herbivores in cotton	FEMS Microbiology Ecology	Missing one or both herbivore categories	No plant-feeding nematode
2019	Zhu et al.	Effect of soil nematode functional guilds on plant growth and aboveground herbivores	Biodiversity Science	Other	Nematodes exposed to root exudates from plants, not directly on the plant

Table S1.3. Plant responses not included in the meta-analysis. Arrows indicate whether the response in insect and nematode treatments was increased or reduced compared to control treatment with insects only. Shaded arrows indicate a significant interaction or error bars between treatments that did not overlap. A forward slash indicates that multiple plant, insect, or nematode species were used in the same paper. A comma represents results from different timepoints within a single paper. For full references see Table S1.

Response	Category	Plant families	# Studies	References	Trend
Jasmonic Acid a. Leaves b. Roots	Plant defense compounds	Solanaceae	2	Guo and Ge 2017 (24h), Kafle et al. 2017	a. ↑; ↑/↑ b. NA; ↑/↑
Glucosinolates (total)	Plant defense compounds	Brassicaceae	2	van Dam et al. 2005 (7 days, 13 days), Hol et al. 2013	↑, ↑; ↓
Protein content (shoots)	Other	Brassicaceae	1	van Dam et al. 2005 (7 days, 13 days)	↑,↑
Phenolics a. Shoots b. Roots-galled c. Roots-nongalled	Plant defense compounds	Brassicaceae, Solanaceae	2	van Dam et al. 2005 (7 days, 13 days), Kaplan et al. 2008b (Phenolic-1, Phenolic-2)	a. ↓,↑; NA/NA, ↑/↑ b. NA, NA; ↑/↑, ↑/↑ c. NA, NA; ↑/↑, ↑/↑
Chlorogenic acid a. Shoots b. Roots—galled c. Roots—nongalled	Plant defense compounds	Solanaceae	1	Kaplan et al. 2008b	a. ↓/↓ b. ↓/↓ c.↑/↓
Nicotine a. Shoots b. Roots—galled c. Roots—nongalled	Plant defense compounds	Solanaceae	1	Kaplan et al. 2008b	a. ↓/↓ b. ↑/↑ c. ↓/↓
Rutin a. Shoots b. Roots—galled	Plant defense compounds	Solanaceae	1	Kaplan et al. 2008b	a. ↑/↓ b. ↑/↑

Table S1.3 (cont'd)

c. Roots—nongalled					c. ↑/↑
Salicylic Acid a. Leaves b. Roots	Plant defense compounds	Solanaceae	2	Guo and Ge 2017 (24h), Kafle et al. 2017	a. ↑; ↓/↓ b. NA; ↓/↑
$\delta^{13}C$ a. Leaf b. Root c. Root:Leaf	Carbon/Nitrogen	Solanaceae	2	Kaplan et al. 2008a, Kaplan et al. 2011	a. 0; ↓ b. ↑; ↓ c. ↑; NA
Cell wall invertase activity a. Leaf b. Root—galled c. Root—nongalled	Other	Solanaceae	2	Kaplan et al. 2008a (galled vs. nongalled not specified), Kaplan et al. 2011	a. ↓; ↑ b. NA; ↓ c. ↑; 0
Vacuolar invertase activity a. Leaf b. Root—galled c. Root—nongalled	Other	Solanaceae	2	Kaplan et al. 2008a (galled vs. nongalled not specified), Kaplan et al. 2011	a. ↑; 0 b. NA; ↑ c. ↑; ↑
Ion current a. Terpenes b. (*Z*)-3-hexenyl acetate c. indole	Plant defense compounds	Malvaceae	1	Olson et al. 2008	a. ↓ b. ↑ c. ↑
Seed capsule number	Other	Solanaceae	1	Schoning and Wurst 2016 (Transient feeding, continuous feeding)	0, ↓
C:N ratio a. Roots b. Shoots	Carbon/Nitrogen	Poaceae, Brassicaceae, Solanaceae	3	Bezemer et al. 2005, Hol et al. 2013, Kafle et al. 2017	a. NA; ↓; ↑/↓ b. ↑/↑; ↑; ↑/↓

Table S1.3 (cont'd)

Amino acids: total a. Leaf b. Phloem	Amino acids	Brassicaceae	2	Hol et al. 2013, Hol et al. 2016 (2 weeks, 5 weeks)	a. ↑; NA, NA b. ↓; 0/↑, ↓/↓
Sugars: total a. Leaf b. Phloem	Other	Brassicaceae	2	Hol et al. 2013, Hol et al. 2016 (2 weeks, 5 weeks)	a. ↑; NA, NA b. ↓; ↓/↓, ↓/↓
Amino acids: individual	Amino acids	Brassicaceae, Poaceae	3	Bezemer et al. 2005, Kutyniok et al. 2014, Hol et al. 2013	Mixed, Mixed, Mixed
Non-nicotine alkaloids a. Shoots b. Roots-galled c. Roots-nongalled	Plant defense compounds	Solanaceae	1	Kaplan et al. 2008b	a. ↓/↓ b. ↑/↑ c. ↓/↓
Heliocides a. Immature leaf b. Mature leaf	Plant defense compounds	Malvaceae	1	Olson et al. 2008	a. 0/↑/↑ b. ↓/↓/↓
Water content (shoots)	Other	Brassicaceae	2	van Dam et al. 2005 (7 days, 13 days), Hol et al. 2013	↓ , 0; ↓
Gossypol a. Immature leaf b. Mature leaf c. Root	Plant defense compounds	Malvaceae	1	Olson et al. 2008	a. ↑ b. ↓ c. ↑
Diterpene glycosides (shoots)	Plant defense compounds	Solanaceae	1	Kaplan et al. 2008b	↑/↑
Hemigossypolone a. Immature leaf b. Mature leaf	Plant defense compounds	Malvaceae	1	Olson et al. 2008	a. ↑ b. ↓
$\delta^{15}N$ a. Leaf b. Root	Carbon/Nitrogen	Solanaceae	1	Kaplan et al. 2011	a. ↓ b. ↑
Glucobrassicin	Plant defense compounds	Brassicaceae	1	Hol et al. 2013	↓

Table S1.3 (cont'd)

Glucoiberin	Plant defense compounds	Brassicaceae	1	Hol et al. 2013	↓
Gluconapin	Plant defense compounds	Brassicaceae	1	Hol et al. 2013	↓
Glucoraphanin	Plant defense compounds	Brassicaceae	1	Hol et al. 2013	↓
Hydroxyglucobrassicin	Plant defense compounds	Brassicaceae	1	Hol et al. 2013	↓
Methoxyglucobrassicin	Plant defense compounds	Brassicaceae	1	Hol et al. 2013	↓
Progoitrin	Plant defense compounds	Brassicaceae	1	Hol et al. 2013	↓
SAG	Plant defense compounds	Brassicaceae	1	Kutyniok and Muller 2012	↑
Sinigrin	Plant defense compounds	Brassicaceae	1	Hol et al. 2013	↓
Glucotropaeolin	Plant defense compounds	Brassicaceae	1	Hol et al. 2013	0

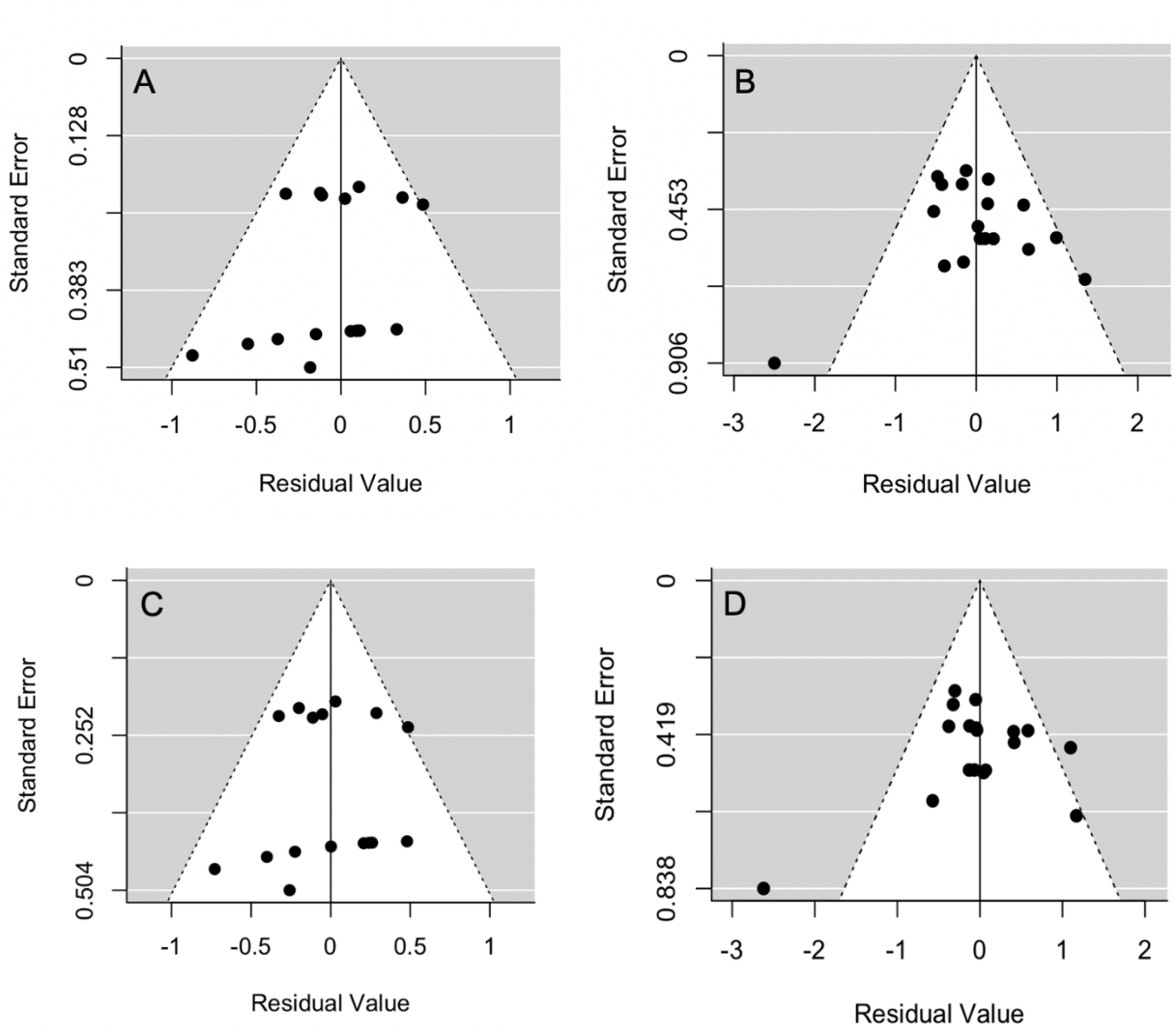
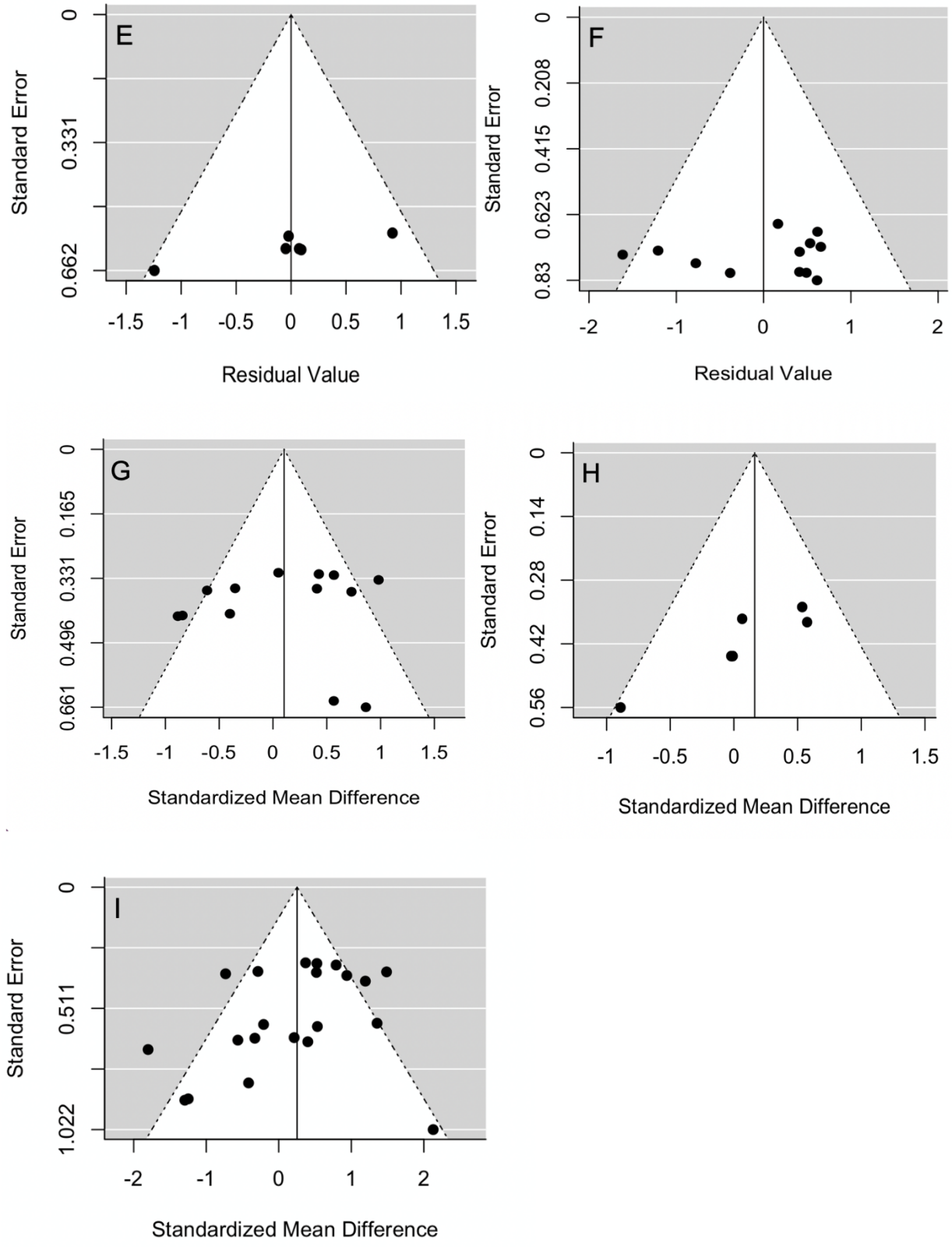


Figure S1.1. Funnel plots for models used in the main text: effect of nematode feeding guild on insect growth (A), effect of nematode feeding guild on insect reproduction (B), effect of plant family on insect growth (C), effect of plant family on insect reproduction (D), Change in carbon (E), change in nitrogen (F), effect of insect presence on nematode egg production (G), effect of insect presence on nematode gall/cyst production (H), and effect of insect presence on total nematodes in roots/soil (I). All funnel plots passed Egger's test for asymmetry in funnel plots except E and H (A: z = -1.2742, p = 0.2026; B: z = -0.0513, p = 0.9591; C: z = -0.6243, p = 0.5324; D: z = -0.3017, p = 0.7629; E: z = -3.1558, p = 0.0016; F: z = -0.3657, p = 0.7146; G: z = 0.0816, p = 0.9350; H: z = -2.2525, p = 0.0243; I: z = -1.7022, p = 0.0887). This indicates that publication bias was only identified in our model of carbon concentration and nematode gall/cyst production.

Figure S1.1 (cont'd)



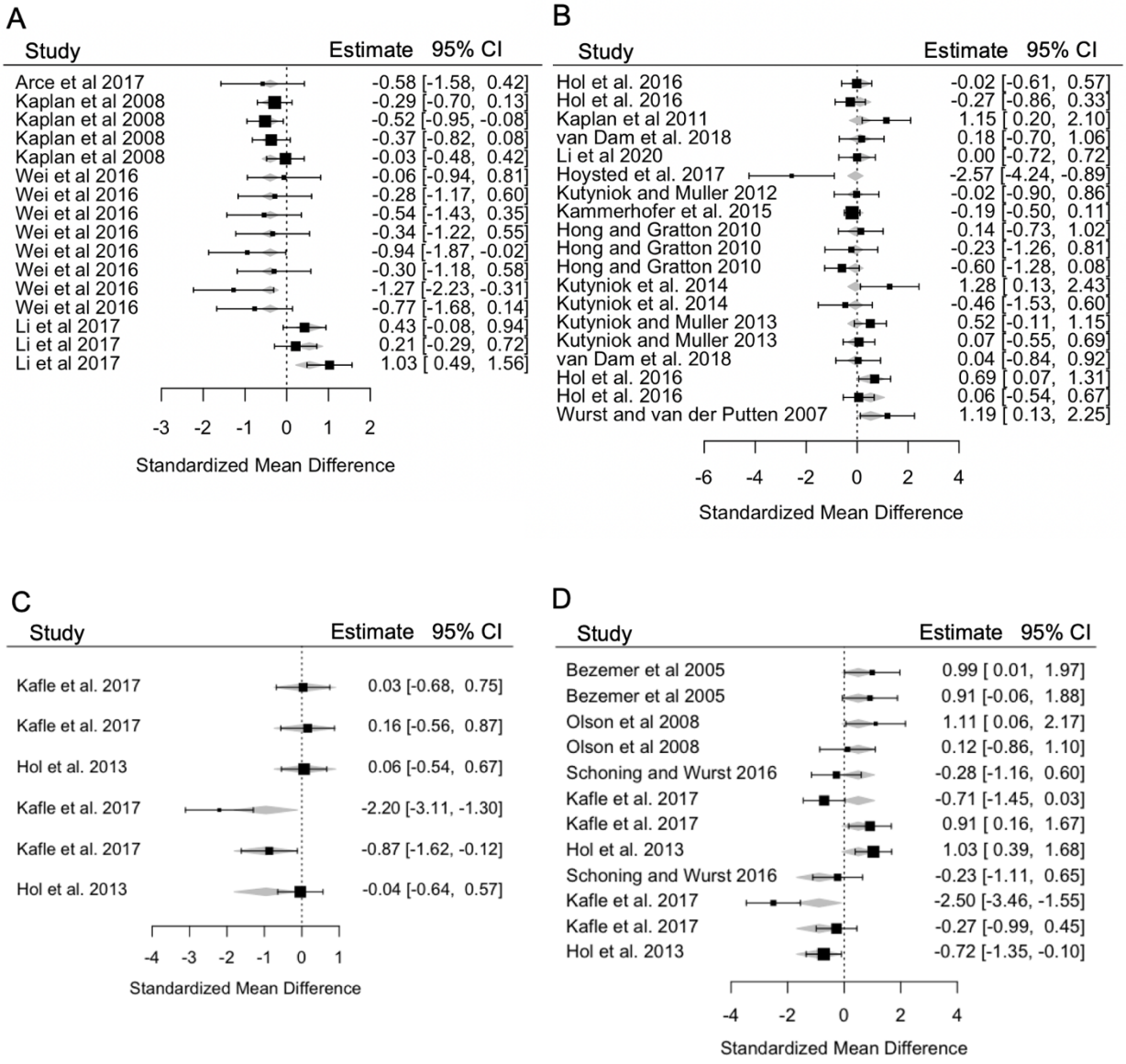
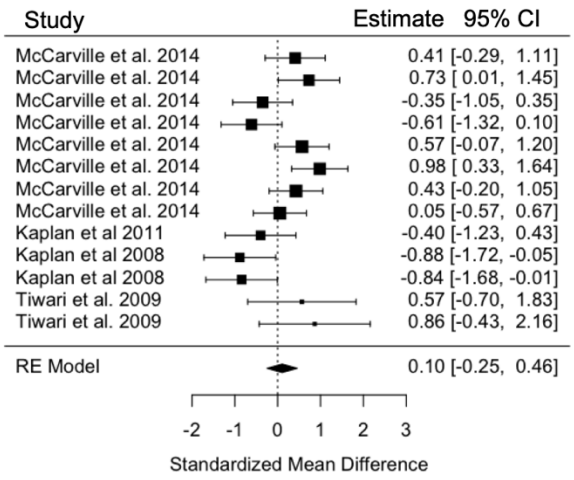


Figure S1.2. Forest plots for models used in the meta-analysis. Standardized mean differences (CI: confidence interval) for response variables are insect growth (A), insect reproduction (B), plant carbon (C), plant nitrogen (D), nematode egg production (E), nematode gall/cyst production (F), and total nematode individuals in the roots and soil (G). See Table S1.1 for details of the studies.

Figure S1.2 (cont'd)

E



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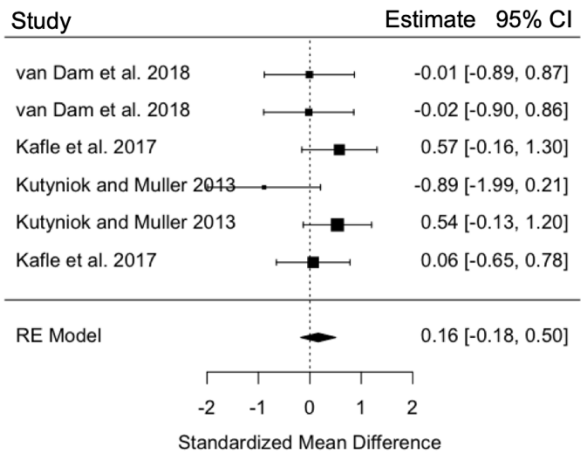

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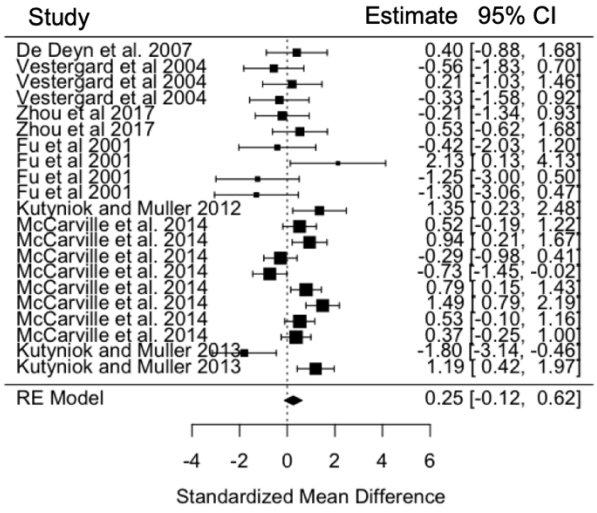


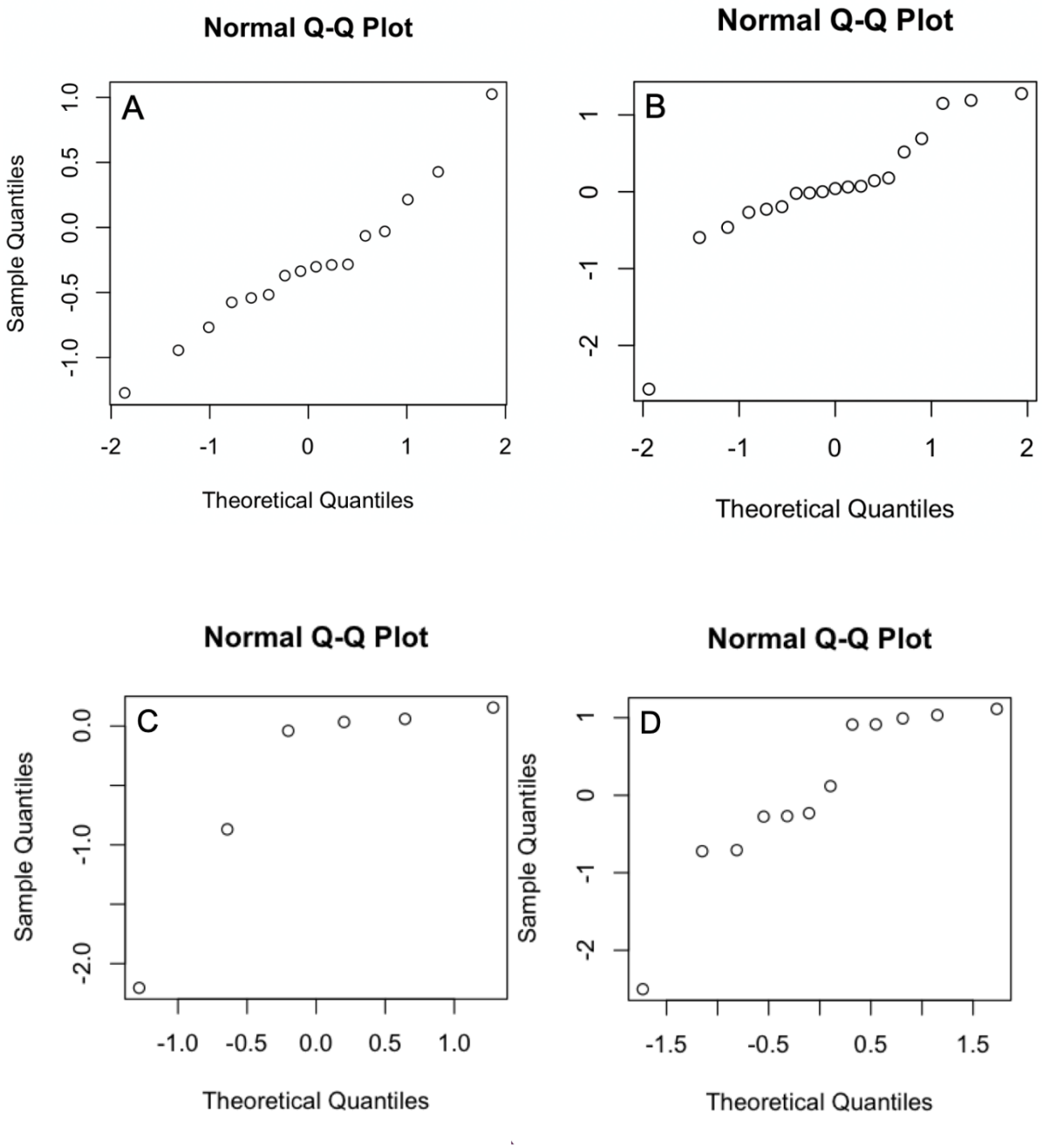
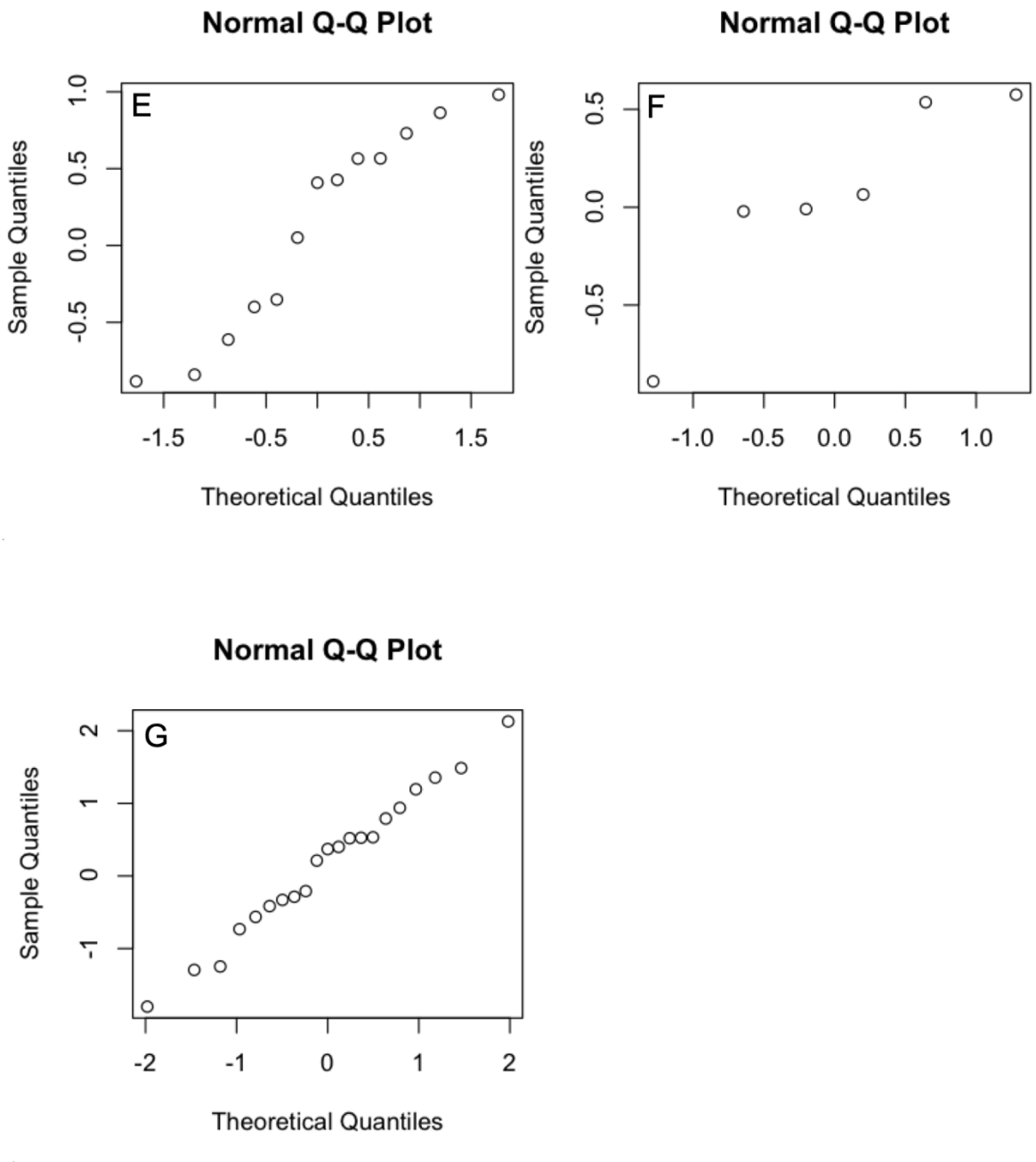


Figure S1.3. Quantile-quantile plots for models used in the main text. Response variables are insect growth (A), insect reproduction (B), plant carbon (C), plant nitrogen (D), nematode eggs (E), nematode galls/cysts (F), and total nematode individuals in roots/soil (G).

Figure S1.3 (cont'd)



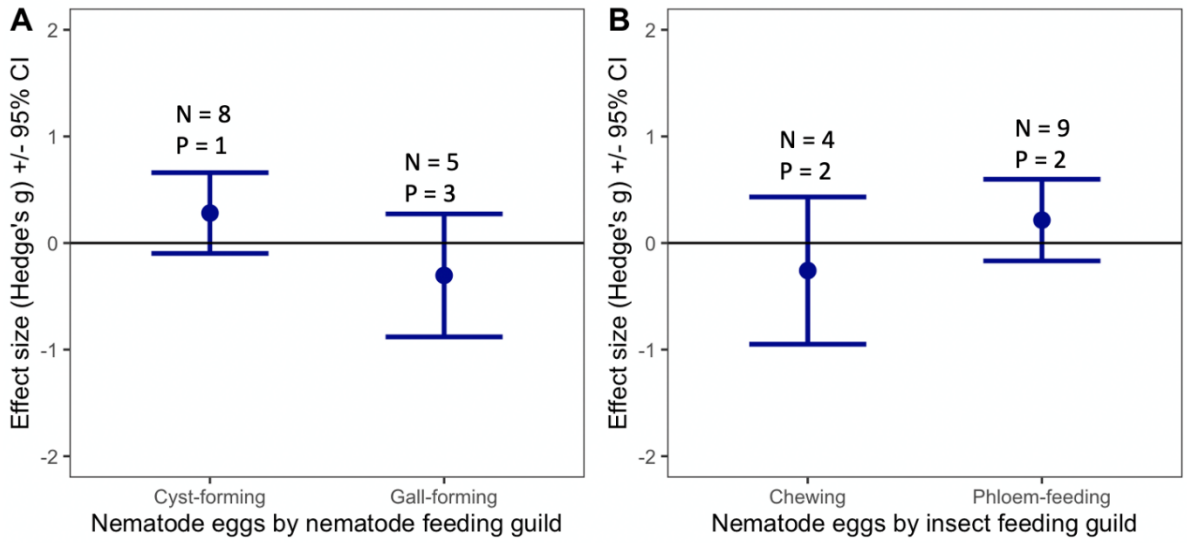


Figure S1.4. Influence of nematode feeding guild (A) and insect feeding guild (B) on nematode egg production in the presence of insects. Response variable is effect size (Hedge's g, standardized mean difference) +/- 95% confidence interval (CI). The effect size compares the response variable on plants with nematode and insect herbivores (treatment plants) to the response variable on plants with nematode herbivores only (control plants). N = number of unique observations included; P = number of unique papers observations are drawn from.

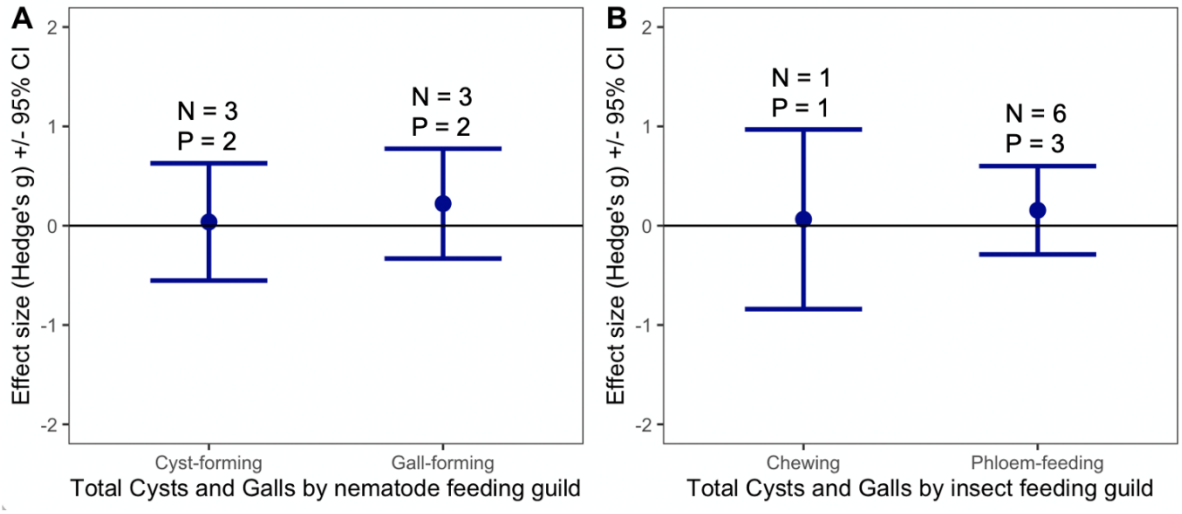


Figure S1.5. Influence of nematode feeding guild (A) and insect feeding guild (B) on nematode cyst and gall number in the presence of insects. Response variable is effect size (Hedge's g, standardized mean difference) +/- 95% confidence interval (CI). The effect size compares the response variable on plants with nematode and insect herbivores (treatment plants) to the response variable on plants with nematode herbivores only (control plants). N = number of unique observations included; P = number of unique papers observations are drawn from.

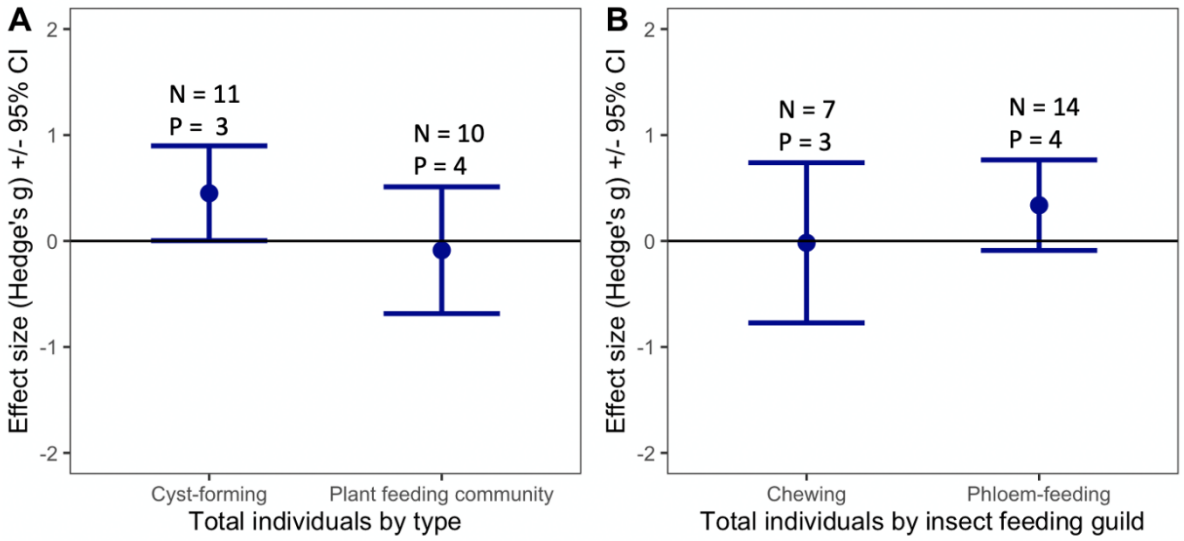


Figure S1.6. Influence of nematode feeding guild (A) and insect feeding guild (B) on nematode abundance in the presence of insects. Response variable is effect size (Hedge's g, standardized mean difference) +/- 95% confidence interval (CI). The effect size compares the response variable on plants with nematode and insect herbivores (treatment plants) to the response variable on plants with nematode herbivores only (control plants). N = number of unique observations included; P = number of unique papers observations are drawn from.

CHAPTER 2:

CONSTITUTIVE LEVEL OF SPECIALIZED SECONDARY METABOLITES AFFECTS PLANT PHYTOHORMONE RESPONSE TO ABOVE- AND BELOWGROUND HERBIVORES

ABSTRACT

Plants defend themselves chemically against herbivory through secondary metabolites and phytohormones. Few studies have investigated how constitutive variation in secondary metabolites contributes to systemic herbivory response. We hypothesized that plants with lower constitutive defenses would induce a stronger phytohormone response to spatially separated herbivory than plants with high constitutive defense. We used growth chamber bioassays to investigate how aboveground herbivory by Colorado potato beetle (*Leptinotarsa decemlineata*, CPB) and belowground herbivory by northern root-knot nematode (*Meloidogyne hapla,* RKN) altered phytohormones and glycoalkaloids in roots and shoots of two lines of wild potato (*Solanum chacoense*). These lines had different constitutive levels of chemical defense, particularly leptine glycoalkaloids, which are only present in aboveground tissues. We also determined how these differences influenced the preference and performance of CPB. The susceptible wild potato line responded to aboveground damage by CPB through induction of jasmonic acid (JA) and 12-oxo-pytodienoic acid (OPDA; a precursor compound to JA). However, when challenged by both RKN and CPB, the susceptible line retained high levels of JA, but not OPDA. Beetles gained more mass after feeding on the susceptible line compared to the resistant line, but were not affected by nematode presence. Belowground, JA, JA-Isoleucine, and OPDA were higher in the resistant line compared to the susceptible line, and demonstrated response to herbivory. In contrast, the susceptible line did not induce phytohormone defenses

belowground. These findings allow us to predict that constitutive level of defense may influence the threshold of herbivory that may lead to plant-mediated effects on spatially separated herbivores.

INTRODUCTION

Plants can respond dynamically to antagonists, such as insect herbivores, that remove their tissues (Gatehouse 2002), or plant-parasitic nematodes that manipulate the plant to create a nutritional sink (Hewezi and Baum 2013, Jones et al. 2013). Plants have a variety of tools at their disposal to minimize the negative impact of herbivory, but one of the most well-studied and important routes is through chemical defense (e.g. Hare 2011, Dyer et al. 2018). In responding to herbivory, plants induce local responses at the site of feeding to quickly deter herbivory, and also upregulate systemic pathways that protect other parts of the plant through the vascular system, phloem, apoplast, or volatile signals (Ruan et al. 2019). Induced defense responses are often initiated by phytohormone pathways such as jasmonic acid (JA), salicylic acid (SA), ethylene (ET), and abscisic acid (ABA) (Caarls et al. 2015, Ruan et al. 2019, Yang et al. 2019; Fig. S2.1). In many cases, these pathways regulate both defense and primary functions such as growth (Yang et al. 2019), and unsurprisingly, are broadly conserved across plant taxa (Meyer et al. 1984, Raskin 1992, Walling 2000; Fig. S2.1).

In addition to interacting with broadly conserved phytohormone pathways, plant antagonists also interact with secondary metabolites, such as glycoalkaloids in solanaceous plants (Zhao et al. 2021) or glucosinolates in Brassicaceae (Textor and Gershenzon 2009) (Fig. S2.1). The quantity and identity of these secondary metabolites can have major impacts on plant resistance to herbivory (Kaiser et al. 2020, Hauri et al. 2021) and can even drive insect speciation and plant-insect community diversity (Richards et al. 2015, Glassmire et al. 2016). Many are

constitutive, or are present in the plant regardless of herbivory (Hartmann 1996). Though levels of these compounds may also increase in response to herbivory (Textor and Gershenzon 2009), plants with high levels of constitutive defense are predicted to have a higher threshold for inducing a significant defense response to herbivory, since they receive relatively less herbivory damage and inducing further defenses is costly (Kessler 2015).

Because phytohormone pathways and taxon-specific plant defenses span both above- and belowground tissues, even herbivores that are spatially separated—root and shoot feeders—can influence each other indirectly through plant chemical changes (Soler et al. 2012, 2013, Wondafrash et al. 2013). However, the outcomes of these interactions are variable and often species-specific (Wondafrash et al. 2013, Soler et al. 2013, Hauri and Szendrei 2022). Although several studies have investigated the effects of feeding guild (such as chewing vs. phloem-feeding) on plant-mediated interactions (van Dam et al. 2018) and thus potential crosstalk between phytohormone pathways (Soler et al. 2013, van Dam et al. 2018), there is a knowledge gap in our understanding of how a plant's constitutive level of secondary metabolites influences the outcome of plant-mediated interactions. Additionally, the outcomes of interactions between herbivorous nematodes and insects are dependent on plant family; one possible explanation for this is variation in specialized, taxon-specific secondary metabolites (Hauri and Szendrei 2022) with unique modes of action (e.g., cardenolide inhibition of the enzyme Na^+/K^+-ATPase (Agrawal et al. 2012), or glucosinolate conversion into isothiocyanates that react with insect protein thiols and amines, leading to loss of function (Jeschke et al. 2016). For example, belowground nematode damage can alter glucosinolate composition (Hol et al. 2013) and quantity (Van Dam et al. 2005) in aboveground tissues, indicating that these compounds play a role in plant-mediated defenses.

Spatially separated herbivores often interact with plant chemical pathways by changing the relative strength of defenses. This can occur because the initial attacker induces systemic pathways, thus leading to a response that is stronger than for local herbivory alone (Fig. S2.1B). Alternatively, an attacker may suppress defense pathways (Fig. S2.1B). This is especially true of plant-parasitic nematodes, which interact intimately with plant defenses by utilizing stylet secretions to negate or alter plant defense responses to form their feeding site (Hewezi and Baum 2013). However, how significantly plants' defense strategy is altered by suppression would likely depend on how much the plant invests in constitutive defense vs. induction. This interference may have a reduced impact on spatially separated herbivores in plants that have high levels of constitutive defense. Thus, we hypothesized that plants with high levels of constitutive defense would show less significant local and systemic (root-to-shoot and shoot-to-root) responses to herbivory than plants with low levels of constitutive defense.

To determine how plants with different levels of secondary metabolites respond to spatially separated herbivores above- and belowground, we performed a set of growth chamber and laboratory experiments using two recombinant inbred wild potato (*Solanum chacoense)* lines that differed quantitatively and qualitatively in glycoalkaloid content. These lines specifically differed in the presence of leptines, which are acetylated glycoalkaloids only present in aerial tissues known to provide resistance to the Colorado potato beetle (*Leptinotarsa decemlineata;* hereafter, CPB) through cell membrane disruption and cholinesterase inhibition (Kaiser et al. 2021). We exposed plants with high and low levels of constitutive defense to the northern root-knot nematode (*Meloidogyne hapla*; hereafter, RKN) which forms galls in the plant root; to CPB, a chewing herbivore; to both; or to neither. We then measured levels of phytohormones and glycoalkaloids in plant roots and shoots, as well as CPB preference and performance when

exposed to different combinations of plant line and RKN presence. These experiments allowed us to answer the following questions: 1) how do root and shoot herbivory, both separately and in combination, influence induction of phytohormone pathways? 2) how does above- and belowground herbivory alter the expression of family-specific secondary metabolites? and, 3) how do plant chemical defense changes in response to belowground herbivory affect an aboveground chewing herbivore? Answering these questions will help us to understand the role of different types of chemical defenses in mediating spatially separated herbivore interactions.

MATERIALS & METHODS

Organisms for experiments

To investigate the effects of leptine on plant-mediated interactions between above- and belowground herbivores, we used two breeding lines generated from a cross between the *S. chacoense* lines USDA 8380-1 and M6 that differed in the presence of leptines I and II (Kaiser et al. 2021). Leptines are acetylated glycoalkaloids only present in aerial tissues known to provide resistance to CPB through cell membrane disruption and cholinesterase inhibition (Kaiser et al. 2021). Line EE501 F5_093_02_05_01 (hereafter, 'susceptible'), contained 0 mg/g dry weight leptine I or II. Line EE501 F5_278_02_01_03 (hereafter, 'resistant'), contained an average of 1.6 mg/g dry weight leptine I and 0.22 mg/g leptine II (Table S5 in Kaiser et al. 2021). Additionally, the susceptible line produced fewer, larger leaves, while the resistant line produced a higher number of smaller leaves; no difference in root structure was observed. Plants were maintained in tissue culture on Murashige and Skoog basal medium with vitamins and sucrose (M5501; Murashige and Skoog salts at 8.8g L^{-1}, 3% sucrose, pH 5.8, and 0.6% plant agar; Murashige and Skoog 1962) at 22°C and 16h:8h L:D cycle for 2 weeks after propagation. At that point plantlets were transplanted to a 50:50 mix of play sand (Quikrete, Atlanta, GA) or all-purpose sand

(KolorScape, Atlanta, GA) and topsoil (Oldcastle Lawn & Garden, Inc., Atlanta, GA) in 9 cm^3 pots. Plants were then maintained in growth chambers at 25°C on a 16:8 L:D cycle and watered *ad libitum*. All plants were fertilized with a 375 ppm solution of 20-20-20 NPK fertilizer (Jack's Professional 20-20-20 fertilizer, JR Peters, Allentown, PA) weekly starting one week post-transplant.

Colorado potato beetles were maintained in a colony initiated with field-collected individuals from the Michigan State University Montcalm Potato Research Center (Lakeview, MI) in May 2020. Beetles were maintained on potato (*Solanum tuberosum*) cv. Atlantic or Russet Norkotah on a 16h:8h L:D cycle at 22-28 °C. Neither cultivar produces leptine; the compound has been found only in certain lines of *S. chacoense* (Sinden et al. 1986). Egg masses for experiments were transferred to Petri dishes where larvae were allowed to hatch and provided *S. tuberosum* leaves prior to use in experiments.

Root-knot nematode colonies were maintained on eggplant (*Solanum melongena* cv. Black Beauty (Burpee, Warminster Township, PA) or tomato (*Solanum lycopersicum*) cv. New Girl (Johnny's Selected Seeds, Winslow, ME) in a 50:50 sand:topsoil mix in Michigan State University's Plant Science Research Greenhouses. Plants were watered *ad libitum* with a 1:20 ratio of water to NPK fertilizer concentrate (200-300 ppm; Jack's Professional 20-20-20 fertilizer). Root-knot nematode eggs were elucidated from host plant roots using a slightly adapted, 1% NaOCl shaking protocol (Hussey and Barker 1973). Eggs were stored in plastic tubes with water after extraction and before inoculation, at approximately 17,000 eggs/ml.

For all experiments, 2-week-old plants in nematode treatments were inoculated with 1,100 RKN eggs per 100 cm^3 soil. Eggs were pipetted into four holes approximately 1 cm deep and 1-2 cm from the plant stem, made with the non-tip end of a fine point Sharpie marker

(Newell Brands, Atlanta, GA) which were then covered with soil; control plants were inoculated with an equal volume of deionized water. Plants were allowed to develop for three weeks before use in experiments to allow for nematode hatching and invasion of the root (Fig. 2.1). A small number of plants of each treatment with belowground damage were stained with acid fuchsin to confirm the presence of galls.

Internal chemistry

We investigated how different types of herbivory (nematode or beetle) influenced plant glycoalkaloid and phytohormone content in roots and shoots. We measured the following phytohormones: jasmonic acid (JA), jasmonic acid isoleucine (JA-Ile), 12-oxo-pytodienoic acid (OPDA), salicylic acid (SA), salicylic acid beta-glucoside (SAG), and abscisic acid (ABA). Jasmonic acid, JA-Ile, and OPDA are all components of the JA pathway, which is typically involved in defense against necrotrophic pathogens (Yang et al. 2019) and wounding due to herbivory (Schilmiller and Howe 2005). OPDA is a JA precursor, and JA-Ile is the biologically active form of JA (Yang et al. 2019). The SA pathway is primarily associated with response to biotrophic pathogens and viruses; SAG is a storage form of SA (Vlot et al. 2009). ABA is involved in drought response and seed development, among other functions (Nakashima and Yamaguchi-Shinozaki 2013).

Plants with and without leptines were grown as described above and exposed to one of four herbivory treatments: no herbivory; aboveground only (CPB); belowground only (RKN); or both (CPB and RKN), for a total of eight treatments with 9-10 replicates (one replicate = one plant) per treatment. Three weeks after nematode inoculation, one 2nd instar CPB was bagged on each plant in an aboveground herbivory treatment and allowed to feed for 24h. All plants were

then transferred to the lab, where beetles were removed. Plant roots were gently washed to remove soil and the entire plant was frozen at -80°C until processing.

Plant tissues were processed for LC-MS analysis according to a modified protocol from Zeng et al. 2011. Approximately 0.07-0.1g frozen leaf tissue from fully expanded leaflets or 0.03-0.1g root tissue was weighed and added to 2 ml polypropylene microtubes (USA Scientific, Ocala, FL) with three 3 mm stainless steel balls (SPEX Sample Prep, Metuchen, NJ) per tube. Aboveground samples typically consisted of 2-5 complete leaflets; root biomass was smaller than aboveground biomass, and samples were often comprised of the complete root system of a plant. Frozen tissue was ground in a pre-frozen bead beater at 30/s until fully ground. Samples were extracted with 1 ml extraction buffer (80:20 v/v methanol:water, 0.1% formic acid, with internal standards SA-13C6, ABA-d6, JA-d5, digitoxin). After incubating at 4°C on a rocking platform for 16h, samples were centrifuged at 4°C for 10 minutes at 14,000 rpm. The supernatant (80 µl) was transferred to high-performance liquid chromatography vials (Restek, Bellefonte, PA) with 250µl inserts (Agilent Technologies, Santa Clara, CA) for phytohormone analysis, and 10 µl was transferred to an HPLC vial containing 990 µL extraction buffer for glycoalkaloid analysis.

Samples were stored at -20°C until processing at the Michigan State University's Mass Spectrometry and Metabolomics Core (East Lansing, MI). Glycoalkaloid samples were analyzed using a Waters Xevo G2-XS Quadrupole-Time-of-flight LC/MS/MS system with a Waters Acquity BEH-C18 UPLC column (2.1x100mm). The machine was operated in positive ion mode. Compounds were eluted using a binary gradient of solvent A (0.1% formic acid in water) and solvent B (acetonitrile) at a flow rate of 0.3 ml min^{-1} at 40°C following a stepwise gradient: 98.0% A, 2.0% B; 0.50 min, 85.0% A, 15.0% B; 5.00 min, 40.0% A, 60.0% B; 7.00 min, 1.0%

A, 99.0% B; 8.00 min, 1.0% A, 99.0% B; 8.01 min, 98.0% A, 2.0% B; 10.00 min, 98.0% A, 2.0% B. Jasmonic acid (JA), JA-Ile, OPDA, SA, SAG, and ABA were analyzed with a Waters Xevo TQ-S triple quadrupole LC/MS/MS system with a Waters Acquity BEH-C18 UPLC column (2.1x50mm). Phytohormones were eluted using a binary gradient of solvent A (0.1% formic acid in water) and solvent B (acetonitrile) at a flow rate of 0.4 ml/min at 40°C following a stepwise gradient: 98% A, 2% B; 0.5 min, 98% A, 2% B; 3 min, 30% A, 70% B; 4 min, 1% A, 99% B; 5 min, 1% A, 99% B, 5.01 min, 98% A, 2%B; 6 min, 98% A, 2% B. MS/MS details for the targeted phytohormone method can be found in Table S2.1. Data were collected with Waters MassLynx software and processed with Waters Quanlynx MS software. Prior to statistical analysis, internal chemistry data were normalized to internal standards (phytohormones: JA-d5, ABA-d6, and SA-$13C_6$; glycoalkaloids: digitoxin) and tissue sample mass.

Preference and performance assays

We used a choice assay to determine how RKN presence influenced CPB larval preference, and no-choice assays to determine how RKN presence influenced CPB larval performance when provided with different plant conditions (susceptible or resistant). The choice assay was performed in metal mesh cages (30 cm^3, Bioquip, Rancho Dominguez, CA). A single larva (5-6 days old) was placed on a Petri dish equidistant between two plants of a single line ('susceptible', N = 22; or 'resistant', N = 25), one inoculated with nematodes and one uninoculated. After 24h, we recorded the larva's location. Larvae not located on a plant after 24h were excluded from the analysis. In no-choice assays, larvae were weighed and randomly assigned to an experimental replicate. For the no-choice assay, a single larva (5-6 days old) was bagged on a plant and allowed to feed for 5 days (susceptible – nematodes: N = 19; susceptible + nematodes: N = 21; resistant – nematodes: N = 24; resistant + nematodes: N = 22). Larvae were

then removed, and weights were recorded. We also visually estimated the aboveground biomass removed by herbivory to the nearest 5% and counted the total number of leaflets and the number of damaged leaflets for each plant to check our estimates. The amount of leaf tissue consumed by beetles was calculated by multiplying the total number of leaflets for a plant by the percent removed by herbivory.

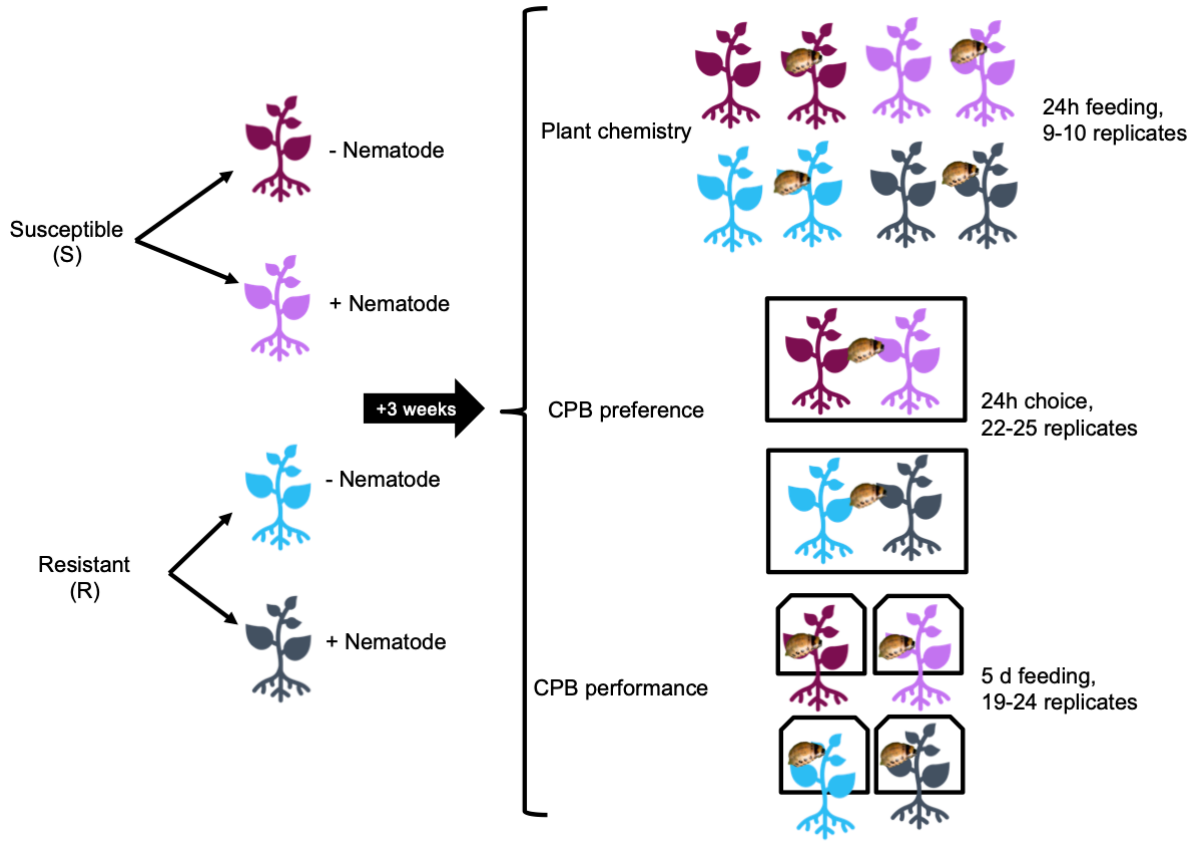


Figure 2.1. Experimental design. Plants of the susceptible (S) and resistant (R) plant lines were grown with and without root-knot nematodes at a rate of 1100 eggs per cubic centimeter of soil for three weeks in the growth chamber. At that point, plants were either used in experiments to assess plant chemistry (phytohormone and glycoalkaloid levels in roots and shoots), CPB preference, or CPB performance. One CPB larva was added per plant for plants in aboveground herbivory treatments.

Statistical analyses

All analyses were performed in R version 4.2.2. (R Core Team 2022). Because we measured a small number of phytohormones but a much larger number of glycoalkaloids (>3500

across all samples), we chose to analyze individual phytohormones and glycoalkaloid composition (including leptine and non-leptine glycoalkaloids). Glycoalkaloid composition by treatment was analyzed with the following functions, all from the package 'vegan' (Oksanen et al. 2022): Permutational multivariate analysis of variances (PERMANOVA) were calculated for models using the 'adonis2' function; dispersion was calculated with the 'vegdist' function (method = 'bray') followed by the 'betadisper' and 'permutest' functions; and pairwise comparisons were performed with the 'pairwise.adonis2' function. For PERMANOVA, leaf data were square root transformed and a Bray-Curtis dissimilarity matrix was created using these values. For both PERMANOVA and dispersion analyses, we evaluated models with plant line, CPB presence, and nematode presence as interactive fixed effects. Because this was not possible with the 'permutest' function, we evaluated each combination of plant line, nematode presence, and CPB feeding as 'treatment'. Nonmetric multidimensional scaling (NMDS) plots were created using the 'metaMDS' function; 95% confidence intervals were calculated with the 'anosim' function in the package 'vegan' (Oksanen et al. 2022).

Individual phytohormone compounds (JA, JA-Ile, OPDA, SA, SAG, and ABA) were analyzed with generalized linear models with nanomoles g^{-1} of compound as the response variable, and line, treatment, or their interaction as fixed effects. Beetle preference data (location after 24h) was analyzed using a χ^2 test, and performance data (change in mass after 1 week of feeding or amount of tissue consumed) was analyzed using linear mixed models with plant line and nematode as additive or interactive fixed effects, and experiment date as a random effect. Pairwise comparisons for performance were calculated with the function 'emmeans'.

RESULTS

Glycoalkaloid composition

Leaves

Ninety percent of the variation in leaf glycoalkaloid composition was due to plant line ($F_{1,57} = 697.99$, $p < 0.01$, $R^2 = 0.90$, Fig. 2.2A). Only about 1% of the variance in glycoalkaloid composition was explained by CPB feeding ($F_{1,57} = 7.64$, $p < 0.01$, $R^2 = 0.01$, Fig. 2.2A). Nematode presence did not influence leaf glycoalkaloid composition ($F_{1,57} = 1.63$, $p = 0.19$, $R^2 < 0.01$, Fig. 2.2A). Additionally, the dispersion—distance from points to centroids—of glycoalkaloid composition differed among treatments ($F_{7,57} = 2.29$, $p = 0.04$, Fig. 2.2B). Once again, this was largely driven by plant line; on average, distance from points to centroids was 37.5% lower for resistant line leaf samples than susceptible line leaf samples ($F_{1,63} = 17.88$, $p < 0.01$), indicating that the glycoalkaloid composition was more similar between resistant samples than susceptible samples.

Roots

Twenty one percent of variation in root glycoalkaloid composition was due to plant line ($F_{1,54} = 17.71$, $p < 0.01$, $R^2 = 0.21$, Fig. 2.2C). Neither CPB feeding nor nematode presence affected root glycoalkaloid composition ($F \leq 2.11$, $p \geq 0.08$, $R^2 \leq 0.02$, Fig. 2.2C). Dispersion did not differ between treatments overall ($F_{7,54} = 1.41$, $p = 0.22$, Fig. 2.2D) nor were there differences by plant line ($F_{1,60} = 2.67$, $p = 0.1$, Fig. 2.2D). The treatment with the highest average distance to the centroid was the susceptible line with nematodes and without CPB, which had an average distance to median of 0.27; if CPB were present, the average distance to the centroid was lowest at 0.11, nearly a 60% decrease (Fig. 2.2D). On the resistant line, the difference in average

distance to centroids between plants with nematodes alone and plants with nematodes and CPB was only 2.56%.

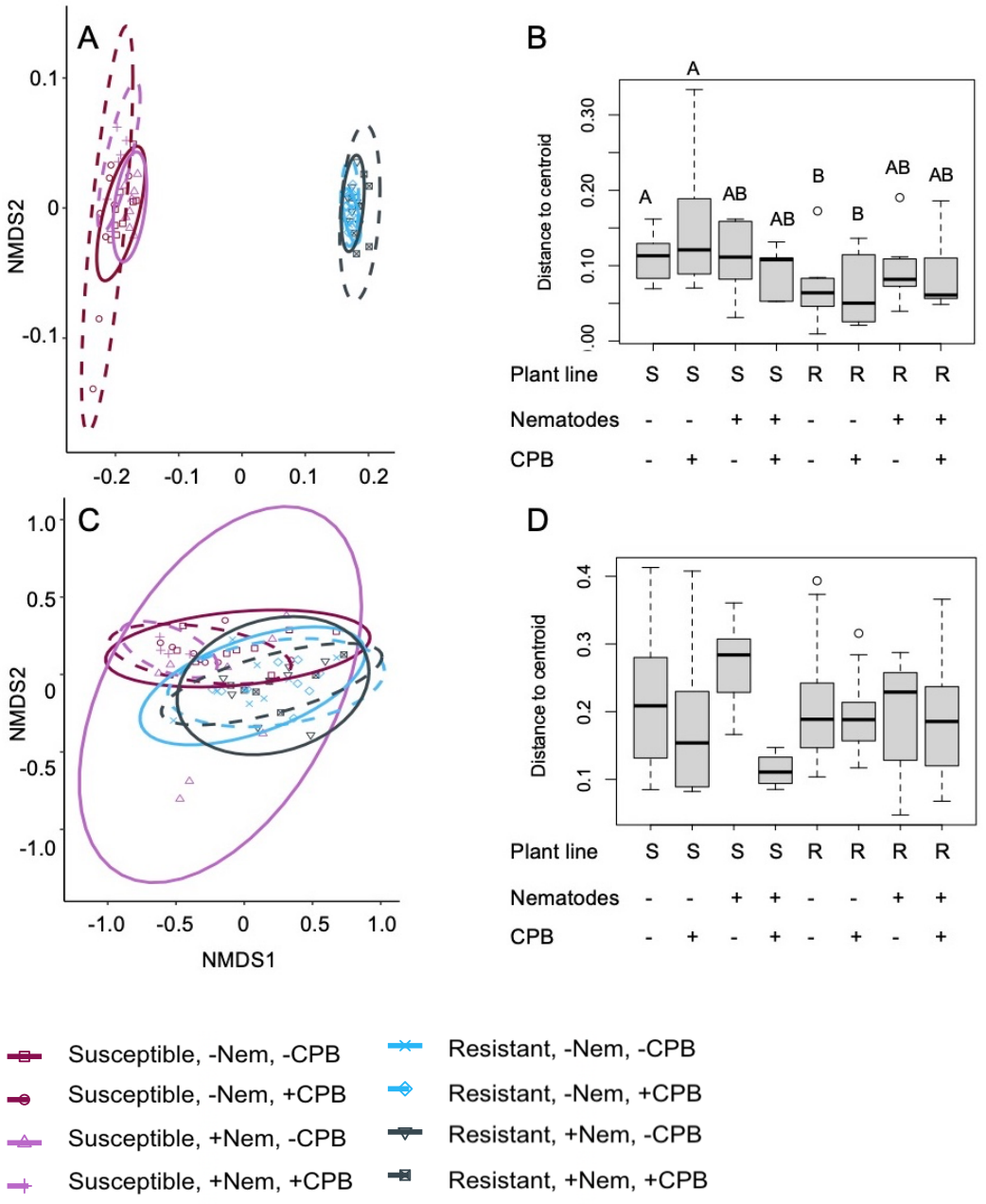


Figure 2.2. Non-metric multidimensional scaling (NMDS) of glycoalkaloid composition by treatment in leaves (A, stress = 0.05) and roots (C, stress = 0.11), and boxplots of distance to centroids for leaves (B) and roots (D). Circles represent 95% confidence intervals of glycoalkaloid composition for each treatment. Treatment includes plant line (Susceptible, S;

Figure 2.2 (cont'd)
Resistant, R), nematode presence (-Nem/+Nem), and CPB presence (-CPB/+CPB) with 9-10 replicates (individual plants) per treatment.

Phytohormone response

Leaves

In general, CPB feeding numerically increased the amount of all compounds in the JA pathway in the leaves—JA, JA-Ile, and OPDA—except JA-Ile in the resistant line, but the increase was more pronounced in the susceptible line (Table 2.1, Fig. 2.3A, B, C). However, the systemic effects of nematode presence only affected the amount of OPDA in the leaves (Table 2.1). While plants without nematodes had a 354% increase in OPDA after CPB feeding, in plants with nematodes there was only a 49% increase; for JA and JA-Ile, this effect did not occur (Table 2.1, Fig. 2.3A-B).

The amount of OPDA, SAG, and ABA in leaf tissue differed between the two plant lines, while SA did not (Table 2.1, Fig. 2.4 A, B, C). For OPDA, this was largely driven by an increase in the susceptible line with CPB herbivory in the absence of nematodes (Fig. 2.3C). The average SAG was higher in the resistant line, at 77.25 nmol g^{-1} leaf tissue, compared to 68.15 nmol g^{-1} in the susceptible line (Fig. 2.3E). In contrast, ABA was 255% higher in the susceptible line with an average of 0.06 nmol g^{-1} compared to 0.02 nmol g^{-1} in the resistant line (Fig. 2.4C), regardless of herbivory.

Roots

In root tissue, the amount of JA, JA-Ile, OPDA, and SAG were higher in the resistant line than the susceptible line (Table 2.1, Fig. 2.3D, E, F; Fig. 2.4D, 1157% higher, 629% higher, 268% higher, and 50% higher in the resistant line compared to the susceptible line, respectively). Local nematode presence reduced JA by 0.02 nmol g^{-1} in the susceptible line and 0.79 nmol g^{-1} in

the resistant line, and JA-Ile by 0.005 nmol g^{-1} in the susceptible line and 0.15 nmol g^{-1} in the resistant line (Table 2.1, Fig. 2.3D, E, F). In contrast, systemic effects from CPB feeding increased OPDA by 0.04 nmol g^{-1} in the susceptible line and 0.08 nmol g^{-1} in the resistant line (Table 2.1, Fig. 2.3F). For compounds in the JA pathway, even when CPB feeding did not elevate compound levels above control levels, there was a numerical increase from treatments with nematodes alone to nematodes and CPB, indicating shoot to root effects (JA, S: 0.08 nmol g^{-1} to 0.12 nmol g^{-1}, 27% increase; JA, R: 0.20 nmol g^{-1} to 0.31 nmol g^{-1}, 173% increase; JA-Ile, S: 0.02 nmol g^{-1} to 0.03 nmol g^{-1}, 56% increase; JA-Ile, R: 0.04 nmol g^{-1} to 0.08 nmol g^{-1}, 104% increase; OPDA, S: 0.08 nmol g^{-1} to 0.12 nmol g^{-1}, 42% increase; OPDA, R: 0.20 nmol g^{-1} to 0.31 nmol g^{-1}, 53% increase).

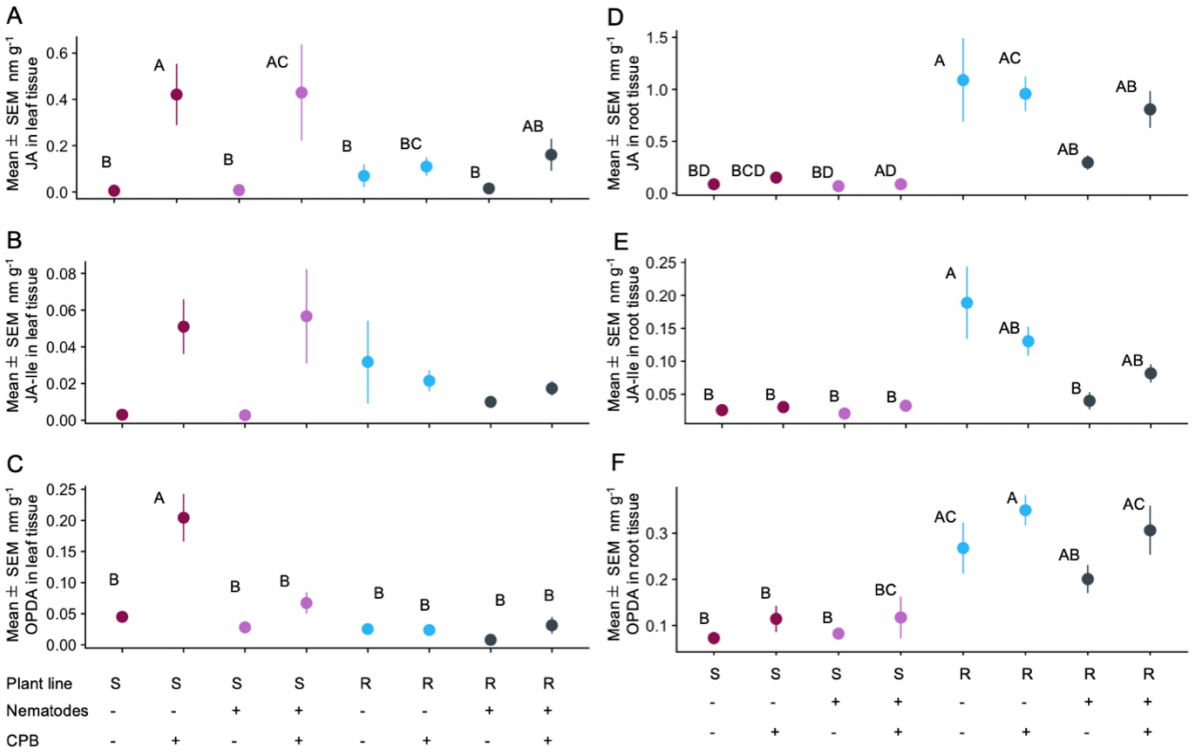


Figure 2.3. Mean ± SEM nanomoles per g leaf tissue A) Jasmonic acid (JA), B) Jasmonic acid isoleucine (JA-Ile), C) 12-oxo-phytodienoic acid (OPDA); and mean ± SEM nanomoles per g root tissue D) Jasmonic acid, E) Jasmonic acid isoleucine (JA-Ile), F) 12-oxo-phytodienoic acid

Figure 2.3 (cont'd)
(OPDA). Letters indicate $p < 0.05$ in pairwise comparisons across all treatments. N = 9 independent replicates per treatment.

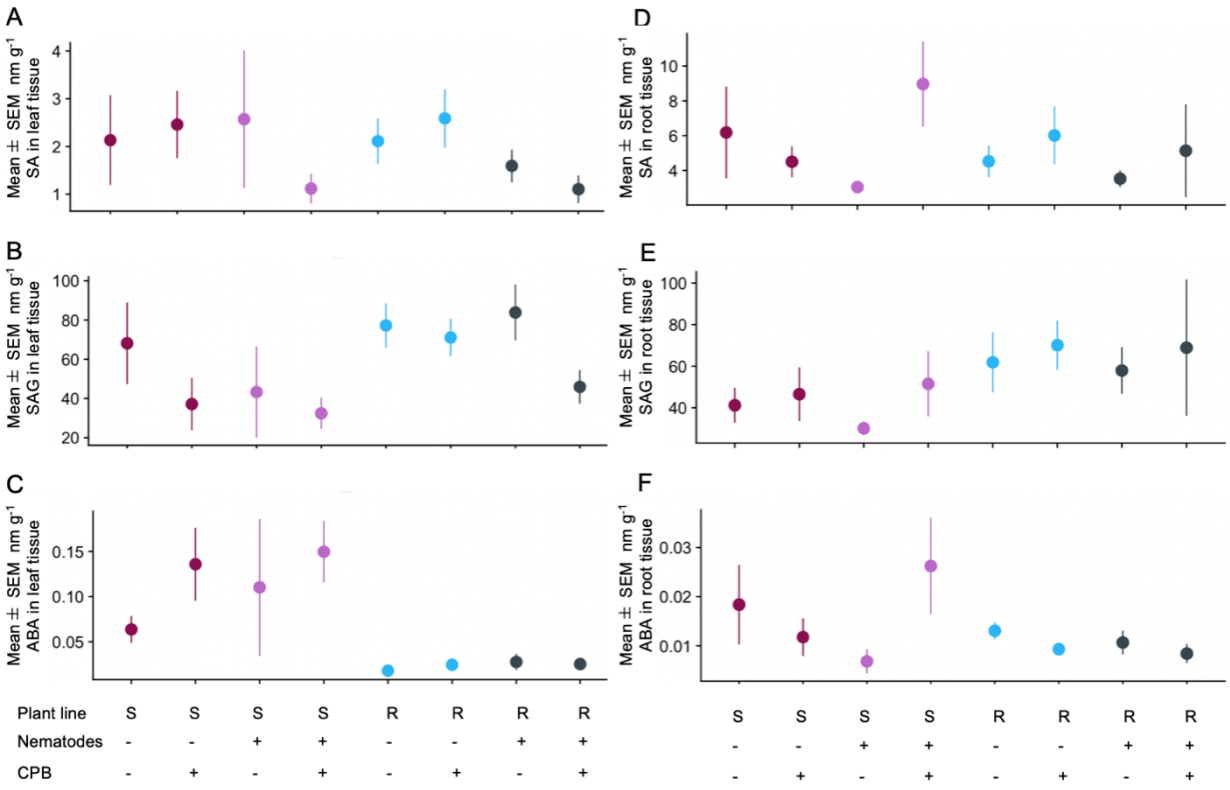


Figure 2.4. Mean ± SEM nanomoles per g leaf tissue A) salicylic acid (SA), B) salicylic acid beta-glucoside (SAG), C) abscisic acid (ABA); and mean ± SEM nanomoles per g root tissue D) SA, E) salicylic acid beta-glucoside (SAG), and F) ABA. N = 9 independent replicates per treatment.

<u>Beetle response</u>

Preference

Beetles had no preference between plants with and without nematodes for the susceptible line ($\chi^2 = 0.05$, df = 1, p = 0.82; Fig. 2.5A). Although they chose nematode-infested plants twice as often as control plants on the resistant line, this difference was not statistically significant ($\chi^2 = 2.33$, df = 1, p = 0.13; Fig. 2.5B).

Performance

Larvae feeding on the susceptible line gained an average of 0.075g (95% CI: 0.056g-0.095g), while larvae feeding on the resistant line only gained an average of 0.047g (95% CI: 0.029g-0.066g), and were on average 37.32% smaller than larvae fed on the susceptible line ($F_{1,81.1} = 7.18$, $p = 0.01$, Fig. 2.5C). Nematode presence did not affect beetle weight change ($F_{1,81}$, $= 0.75$, $p = 0.39$, Fig. 2.5C). Plants of the resistant line had an average of 84.6 leaflets per plant (95% CI: 76.88-93.05), while plants of the susceptible line had an average of 45.80 leaflets per plant (95% CI: 37.90-54.27). Beetles did not consume different amounts of tissue on the two plant lines ($F_{1,86.1} = 0.54$, $p = 0.46$), nor did nematode presence affect their consumption ($F_{1,86.1} = 0.04$, $p = 0.83$).

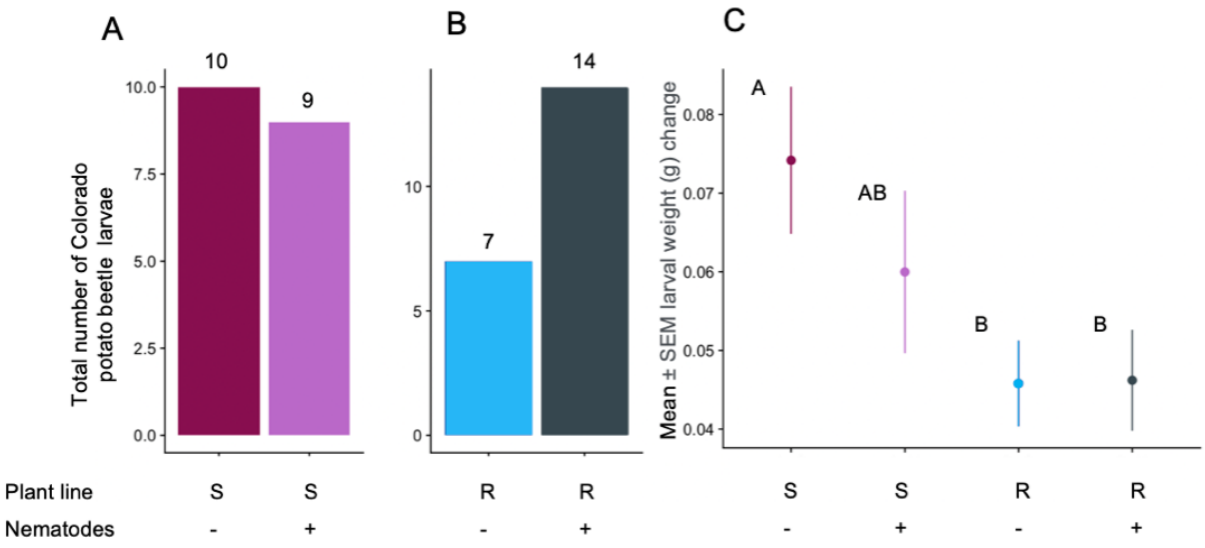


Figure 2.5. Colorado potato beetle (CPB) response to plant and nematode treatments. (A) CPB preference for susceptible plants with and without root-knot nematodes (RKN). Numbers above bars indicate number of CPB larvae found on plants of that treatment after 24h in choice assays. (B) CPB preference for resistant plants with and without RKN. Numbers above bars indicate number of beetle larvae found on plants of that treatment after 24h in choice assays. (C) CPB mass change after one week of feeding on susceptible and resistant plants with and without RKN presence. A single larva (5-6 days old) was bagged on a plant and allowed to feed for 5 days (susceptible – nematodes: N = 19; susceptible + nematodes: N = 21; resistant – nematodes: N = 24; resistant + nematodes: N = 22). Letters indicate $p < 0.05$ in pairwise comparisons.

Table 2.1. Model output (F- and p-values) for plant hormones in the leaves and roots of two *Solanum chacoense* breeding lines that were damaged by *M. hapla* nematodes (RKN) and/or Colorado potato beetle (CPB). Significant p-values are bolded (α = 0.05).

	Plant line		**Nematode**		**CPB**		**Line × Nematode**		**Line × CPB**		**CPB × Nematode**	
Leaves	F	p	F	p	F	p	F	p	F	p	F	p
JA[1]	3.69	0.06	0.26	0.61	22.85	**< 0.01**	0.04	0.85	10.32	**< 0.01**	0.33	0.57
JA-Ile[2]	0.18	0.67	1.07	0.31	6.97	**0.01**	0.41	0.52	9.47	**< 0.01**	0.51	0.48
OPDA[3]	34.38	**< 0.01**	13.95	**< 0.01**	27.00	**< 0.01**	10.06	**< 0.01**	19.87	**< 0.01**	3.77	0.06
SA[4]	0.26	0.62	1.19	0.28	0.12	0.73	0.41	0.53	0.16	0.69	1.35	0.25
SAG[5]	4.53	**0.04**	0.86	0.36	3.64	0.06	0.14	0.71	0.00	0.95	0.11	0.74
ABA[6]	12.33	**< 0.01**	0.40	0.53	1.30	0.26	0.22	0.64	1.26	0.27	0.16	0.69
Roots												
JA	26.69	**< 0.01**	4.27	**0.04**	0.49	0.49	2.78	0.10	0.17	0.68	1.46	0.23
JA-Ile	22.89	**< 0.01**	9.06	**< 0.01**	0.11	0.74	6.81	**0.01**	0.30	0.58	2.46	0.12
OPDA	53.79	**< 0.01**	1.41	0.24	6.56	**0.01**	1.69	0.20	1.07	0.31	0.04	0.85
SA	0.12	0.73	0.34	0.56	1.33	0.25	0.10	0.75	0.01	0.91	1.98	0.16
SAG	4.88	**0.03**	0.18	0.67	0.09	0.34	0.01	0.91	0.01	0.93	0.16	0.69
ABA	1.69	0.20	0.32	0.57	0.00	0.98	0.00	0.95	1.09	0.30	3.91	0.053

[1]Jasmonic acid; [2]Jasmonic acid isoleucine; 3 12-oxo-phytodienoic acid; [4]Salicylic acid; [5]Salicylic acid beta-glucoside; [6]Abscisic acid

DISCUSSION

We examined the effects of above- and belowground herbivory on two lines of a wild potato relative, *Solanum chacoense*, one resistant to herbivory due to high levels of glycoalkaloid constitutive defenses and a susceptible line with lower levels of glycoalkaloids. We found that the susceptible line responded to aboveground CPB damage through induction of JA and OPDA, a JA precursor (Ruan et al. 2019), while the resistant line did not differ in levels of JA, OPDA, or JA-Ile, a biologically active form of JA (Ruan et al. 2019) between herbivory treatments. However, when challenged concurrently by RKN and CPB, the susceptible line retained high levels of JA but not OPDA. Consistent with our hypothesis, the susceptible line exhibited root-to-shoot effects aboveground for OPDA, although not for other compounds. In contrast, the resistant line exhibited no root to shoot effects. Beetle performance reflected the plant defense response, with higher performance on the susceptible line with a numerical (though not statistically significant) decrease in mass change on plants with nematodes, while there was no difference in beetle mass on plants of the resistant line. Belowground, JA, JA-Ile, and OPDA were higher in the resistant line compared to the susceptible line, and contrary to our hypothesis, we did not see any shoot-to-root effects in the susceptible line. Previous studies have found correlated local expression of phytohormones and secondary metabolites following insect herbivory (Robert et al. 2019); our results suggest that prior to herbivory, high constitutive levels of specialized secondary metabolites can result in systemic elevation of related phytohormones, while plants with low constitutive defenses may induce a response primarily towards the most damaging herbivores.

While we observed changes in JA-pathway compounds, levels of ABA, SA, and SAG did not differ between herbivory treatments. Similarly, a study in *Arabidopsis thaliana* found no

change in ABA after inoculation with *Heterodera schachtii* (Kammerhofer et al. 2015). Additionally, lack of induction may represent an effective plant defense strategy, as exogenous application of ABA results in increased nematode parasitism by *Hirschmanniella oryzae* (Nahar et al. 2012). In contrast, functional JA and SA pathways were required for plants to mount chemical defense against nematodes (Nahar et al. 2012). However, SA pathway response in *Solanum* species in response to root knot nematodes is mixed. Previous studies investigating Southern root knot nematode (*Meloidogyne incognita)* in tomato (*Solanum lycopersicum*) found both suppression of leaf and root SA (Kafle et al. 2017) or elevated SA in leaf tissues (Guo and Ge 2016) as a result of nematode infection. In *A. thaliana*, no changes were found in SA, SAG, or JA in independent or simultaneous feeding by *Brevicoryne brassicae* and *H. schachtii* on *A. thaliana* (Kutyniok and Muller 2012). It is likely that the timing of our experiment did not capture the window where SA defenses are most effective: SA defenses (as well as JA pathway defenses) are effective against initial root infection by gall nematodes, while JA defenses better prevent development in the galling stage (Martínez-Medina et al. 2017). Because nematodes in our experiment had initiated gall formation by the time we collected phytohormones, any SA response that did occur would likely have subsided.

In our system, one of the main differences between the susceptible and resistant lines was the presence of leptine glycoalkaloids (Kaiser et al. 2021). These acetylated glycoalkaloids reduce CPB herbivory compared to non-acetylated glycoalkaloids, such as α-solanine and α-chaconine, present in commercial potatoes (Sinden et al. 1986, Kaiser et al. 2020, 2021). However, they are only produced in aboveground tissues (Kaiser et al. 2021). This difference was apparent in our samples: the variability in glycoalkaloid composition was greater aboveground than belowground, and beetle performance was reduced in the resistant line

regardless of nematode presence. An important regulator of the glycoalkaloid signaling pathway in other solanaceous plants is the COI1 gene, which is downstream of the JA signaling pathway (Cárdenas et al. 2016, Montero-Vargas et al. 2018, Zhao et al. 2021). Activation of the JA signaling pathway is one possible explanation for the differences in root responses between the susceptible and resistant lines. While leptine glycoalkaloids are only produced in the shoots, synthesis relies on signaling from an activated JA pathway, which is systemic (Zhao et al. 2021). Therefore, the resistant line—which produces more leptine glycoalkaloids—had higher levels of JA, JA-Ile, and OPDA in root tissues compared to the susceptible line, which had relatively low levels of constitutive glycoalkaloids in comparison. As a result, resistant plants had higher levels of constitutive defense belowground as well, as evidenced by higher levels of JA-pathway compounds in control plants. Future research could test this mechanism with grafting experiments, combining resistant scions with susceptible rootstock, to determine whether aboveground leptine production induces resistance in roots.

The differences in levels of JA-pathway compounds may also have influenced root-to-shoot vs. shoot-to-root effects. In our experiments, we only observed root-to-shoot effects in the case of OPDA in the susceptible line, which contrasts with a previous meta-analysis that showed belowground herbivory typically induced root and foliar defenses to a similar extent, while leaf herbivory does not typically induce a response in the roots (Kaplan et al. 2008). While OPDA was elevated after CPB herbivory on nematode-free plants, there was no change after CPB herbivory on plants with nematodes, although JA levels remained high. Because OPDA is a precursor to JA, it is possible that the presence of RKN limited the plant's ability to upregulate the phytohormone pathway, but that JA was still produced from OPDA that existed in the plant prior to nematode infestation. Long-term, this reduction in OPDA could inhibit the plant's ability

to effectively defend against CPB aboveground. Previous studies have shown a variety of aboveground JA responses to RKN infection, including suppression of *Arabidopsis thaliana* leaf defenses after infection by *Meloidogyne incognita* (Hamamouch et al. 2011) and RKN enhanced JA-marker genes in aboveground tissues of *Brassica nigra* (van Dam et al. 2018). The JA-pathway is critical for defense against RKN colonization, and due to higher constitutive levels of JA in the roots, the resistant line was likely more difficult for nematodes to invade than the susceptible line. OPDA is known to provide resistance to RKN in *Arabidopsis* (Gleason et al. 2016), and *M. incognita* effector protein *MilSE5* interferes with the JA signaling pathway early in infection to promote successful parasitism (Shi et al. 2018). There was evidence for this type of interference in the resistant line; the presence of nematodes alone generally caused a numeric, though only statistically significant for JA-Ile, reduction in JA-pathway compounds in the root. However, these compounds increased to near control levels when CPB feeding occurred simultaneously, even though CPB feeding alone did not typically elevate levels of root JA-pathway compounds, indicating shoot-to-root effects. In soybean roots, infection by the soybean cyst nematode, *Heterodera glycines*, caused a systemic induction of JA, but JA-controlled defenses were suppressed locally (Ithal et al. 2007a, 2007b, Wondafrash et al. 2013). A similar mechanism could explain a reduction in JA-pathway compounds with RKN alone, but an increase as systemic JA induction by CPB feeding overwhelms local suppression.

Systemic effects from plant-mediated interactions are also modified significantly by timing, which we did not investigate. A meta-analysis suggests that belowground herbivores only promote aboveground herbivore success if they were introduced simultaneously, whereas aboveground herbivores only affected belowground feeders when they were introduced first (Johnson et al. 2012). In our study, we introduced RKN as eggs before CPB, with the beetle

larvae added approximately when second-stage juvenile nematodes were initiating galls. For our study system, however, it seems that the strength of herbivory may have overwhelmed the sequence of arrival effects. Colorado potato beetles are defoliators of potato (Alyokhin 2009) and its wild relatives (Sinden et al. 1986), and likely represented a more immediate threat to plant survival than RKN, which damages roots and tubers but rarely causes plant death (Tan et al. 2009). Invertebrate herbivores typically prefer young plants (Kursar and Coley 2003, Boege and Marquis 2005), and plants typically invest more defensive compounds in young compared to old leaves (Gershenzon and Ullah 2022). This was recently demonstrated for family-specific secondary metabolites (glucosinolates) within *Arabidopsis thaliana* (Hunziker et al. 2021). In keeping with optimal defense theory, susceptible plants may have induced a greater response to an aboveground attacker that has the capability to completely destroy the plants. For resistant plants, there was little aboveground response; damage may not have reached a threshold necessary to induce defenses, which has been shown for other plant defenses, such as silicon (Hartley and DeGabriel 2016).

In conclusion, varying the level of family-specific secondary metabolites in plants may partially explain why outcomes of plant-mediated interactions may vary by plant family. Although it is well established that species identity is critical to understanding plant-mediated interactions (e.g. Kaplan et al. 2009, Soler et al. 2013, van Dam et al. 2018), our results will help fine-tune predictions about when an individual species will affect spatially separated herbivores. High constitutive levels of specialized metabolites alter the threshold of damage necessary to induce systemic phytohormone pathways in response to local herbivory. Whether or not a plant reaches that herbivory threshold may in effect alter the timing of the interaction as perceived by organisms feeding on spatially separated parts of the plant. However, maintaining high levels of

specialized compounds is reliant on systemic phytohormone pathways; this may offer an opportunity to plants to overwhelm local suppression by invaders or herbivores. Future studies that investigate the effects on belowground herbivores can help determine if selecting for crop varieties with high levels of constitutive defense—even aboveground only—could be deployed in an agricultural context, where belowground damage is less consistent and control options are limited.

ACKNOWLEDGEMENTS

We thank R. Rantz and S. Kaur for help in the greenhouse and lab, and M. Hufnagel for assistance with tissue culture propagation of plant lines. We thank E. Cinto-Mejia, N. Constancio, N. Carvajal, D. Dillard, L. Marmolejo, J. Zavalnitskaya, M. Eeraerts, and J. Ali for valuable comments and feedback. This work was supported by the United States Department of Agriculture (USDA) National Institute of Food and Agriculture (NIFA) Predoctoral fellowship (project number 2021-09359), C.S. Mott Predoctoral Fellowship, and the Sustainable Michigan Endowed Project.

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APPENDIX 2

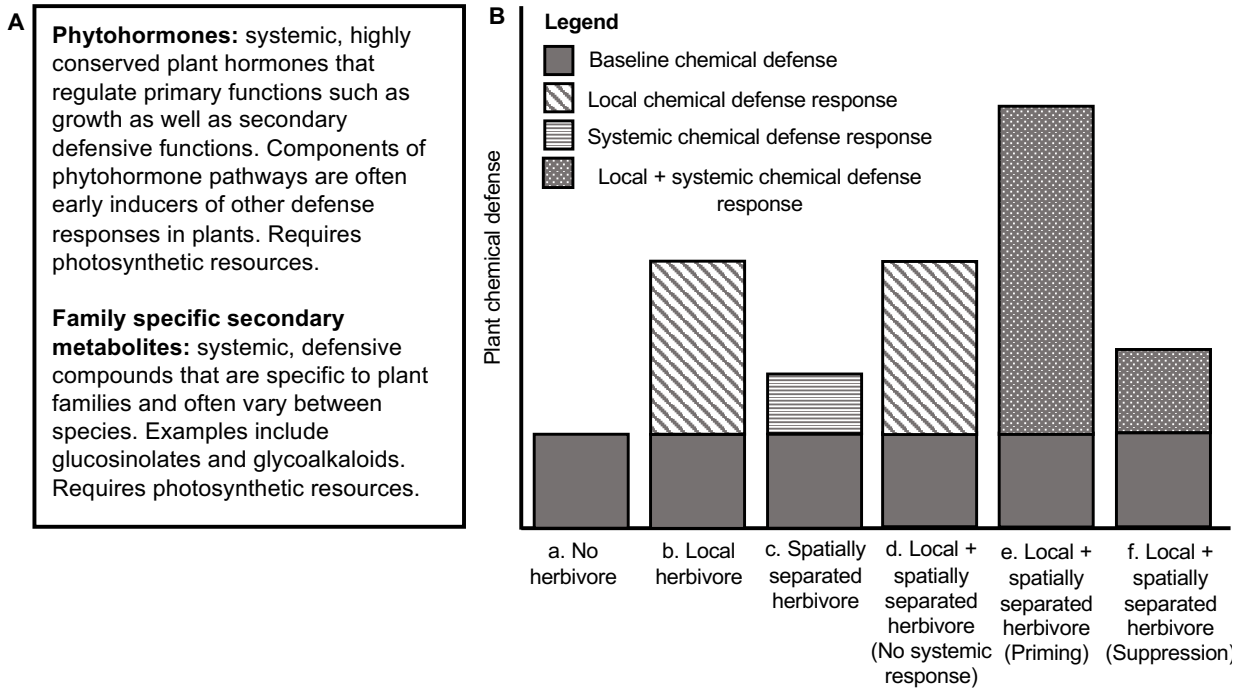

Figure S2.1. Components of plant chemical defense influencing spatially separated herbivores. A) Description of phytohormone and family-specific components of plant defense. B) Possible outcomes of local and spatially separated herbivory on plant defense. Plants can respond locally or systemically, or in both ways, to herbivory. Local response is indicated by a change in plant chemical defenses in the same tissues where the herbivory is occurring compared to a plant without herbivory (a): for example, an increase in jasmonic acid in leaf tissues in response to aboveground beetle herbivory (b). Responses to spatially separated herbivores are expected to increase plant defense compared to (a), a change in glycoalkaloid composition in leaves while the roots are damaged by nematodes (c). On plants with multiple herbivores, a response that is no different from a local response (b) would indicate a lack of systemic effects (d). Systemic response is indicated by a difference between the multiple herbivore state and the local herbivore state, either enhancing the response to a single local herbivore, such as through priming (e); or a dampened response to a local herbivore, through suppression of defense pathways (f). For example, in plants with both plant-parasitic nematodes and beetles aboveground, a reduction in plant JA production in leaf tissue following beetle herbivory would indicate a systemic effect (root to shoot) of nematodes.

Table S2.1. MS/MS details for targeted phytohormone method.

Compound	Parent Ion	Daughter Ion	Cone Voltage	Collision Energy
SA	137.1	92.8	25	13
SA-13C6	143	98.9	25	13
JA	209.2	58.8	20	14
JA-d5	214.03	61.9	20	14
ABA	263.2	153.1	25	10
ABA-d6	269.2	159.1	25	10
JA-Ile	322.2	130.1	34	22
SAG	299.1	137	28	16
OPDA	291.2	165.1	46	22

CHAPTER 3:

BELOWGROUND HERBIVORY DIFFERENTIALLY ALTERS PREFERENCE AND PERFORMANCE OF AN ABOVEGROUND PARASITOID AND GENERALIST PREDATOR

ABSTRACT

While belowground herbivory often negatively affects parasitoid preference and performance on aboveground herbivores, little is known about how generalist predators respond and perform in these systems. Because generalists typically display reduced cue specificity compared to parasitoids and are not constrained by the host for development, we expected that parasitoids would prefer aphid hosts on plants without belowground damage, and would parasitize aphids on those plants at a higher rate. In contrast, we expected that the generalist lady beetle would not distinguish between aphids on either plant. We investigated these questions using a focal system of corn aphids (*Rhopalosiphum maidis*), the convergent lady beetle (*Hippodamia convergens*), and the parasitoid *Aphidius colemani* on maize (*Zea mays*). Specifically, we determined how the presence of Western corn rootworm (WCR) larvae altered aphid population growth, consumption by predators, parasitism rates, and natural enemy preference. We also analyzed how the presence of WCR and aphids altered aboveground maize volatile blends after 24h and 1 week of aphid herbivory. Feeding by WCR larvae belowground reduced aphid population size aboveground after 2 weeks of feeding. In contrast to our predictions, *A. colemani* produced equal number of mummies per female between the two treatments, while lady beetles consumed more aphids on control plants. However, *A. colemani* preferred plants with aphids alone over control plants after 24h of aphid feeding, but did not distinguish between other pairwise comparisons, while *H. convergens* did not distinguish

between different herbivory treatments at 24h or 1 week in choice assays. Analysis of aboveground volatiles demonstrated that at both timepoints the combination of above- and belowground herbivory explained more of the variation in volatile blends than either above- or belowground herbivory alone. While most previous work has focused on the effect of belowground herbivory on parasitoids, our results demonstrate the importance of considering generalist predators as well and suggest that better understanding the effects of natural enemies in combination could yield a more accurate understanding of population dynamics in systems with multiple herbivory.

INTRODUCTION

Natural enemies face a complex foraging environment and rely on a broad range of cues to locate prey (Pervez and Yadav 2018, Aartsma et al. 2019). Some of the most important cues are herbivore-induced plant volatiles (Hare 2011). When attacked, plants release a specific blend of compounds that can influence foraging of predators (Takabayashi and Shiojiri 2019), parasitoids (Aartsma et al. 2019), herbivores (Clavijo McCormick et al. 2012, Silva and Clarke 2020), as well as influence the defenses of neighboring plants (Karban et al. 2006). Additionally, volatile blends produced by plants when they are attacked by a single herbivore are often distinct from those produced when the plants are under attack by multiple species (Dicke et al. 2009, Clavijo McCormick et al. 2012). This is true even when the herbivores are feeding on spatially separated organs such as roots and shoots (Rasmann and Turlings 2007, Dicke et al. 2009), creating a potentially difficult environment for natural enemies, where they may be unable to accurately locate prey.

Parasitoids often seek a specific volatile blend, which can lead to avoidance of plants with belowground herbivory due to the presence of repellent compounds that are not present

when a single prey species feeds alone on a plant (Dicke et al. 2009). In a field context, the presence of belowground herbivory in neighboring plants can also increase parasitoid foraging efficiency, possibly because it creates a more contrasting volatile blend that can help parasitoids locate hosts without searching on plants without hosts (Soler et al. 2007). While the vast majority of studies investigating the effects of belowground herbivory on aboveground tritrophic interactions use a focal system of parasitoids (A'Bear et al. 2014; but see Johnson et al. 2013, which reported combined predator and parasitoid abundance), generalist predators inevitably interact with these complex blends as well. However, we do not yet understand how insect predators respond to volatile blend changes resulting from simultaneous aboveground-belowground herbivory. Predators feed on a wide range of species from different feeding guilds, therefore plant semiochemicals—compounds which elicit a response in other organisms—may influence parasitoids and predators differently (Clavijo McCormick et al. 2012). Furthermore, in a field study where plants were challenged with combinations of above- and belowground herbivory, predators and parasitoid responses were grouped and natural enemies were found to respond positively to population size (Johnson et al. 2013). Whether higher populations occur on plants with or without herbivory, however, is highly system specific (Soler et al. 2012, 2013, Wondafrash et al. 2013, Hauri and Szendrei 2022). Thus, if parasitoids respond to specific blends while predators respond to higher herbivore numbers, predators may exhibit a less consistent response than parasitoids.

Additionally, predators and parasitoids may perform differently once they have located hosts. Belowground feeding can negatively influence an aboveground insect by reducing body size or increasing development time and these negative effects often cascade up to parasitoids (Soler et al. 2005, Li et al. 2017). In contrast, reduced body size of the prey may not present a

challenge for predators, where smaller herbivores often mount a less successful defense against predation (Cogni et al. 2002). Predators may also consume more small-bodied prey items to fill their nutritional needs. For example, lady beetles tend to consume more individuals when they are a smaller species (Soares et al. 2004) or earlier life stage (Xia et al. 2003).

Lastly, it is possible that foraging differences between natural enemies when plants are facing multiple herbivores may have significant effects on population dynamics of their herbivore prey. Preferences for volatile blends of plants with a single herbivore over multiple herbivory could lead to reduced predation pressure on plants with above- and belowground herbivory. Despite the fitness cost of alate morphs, locating predator-free space can offset the cost of producing alates to aphid population growth (Ríos Martínez and Costamagna 2017). Similarly, reduced predator risk could provide a counterweight to the nutritional or defensive cost incurred by herbivores feeding on plants with belowground herbivory. If natural enemies with different foraging strategies show contrasting preferences, predation pressure could be more evenly distributed among plants with and without belowground herbivory, reducing the difference in herbivore population size on different plants. These preferences could also change over time, as some volatile compounds used by natural enemies for prey location such as GLVs are released immediately after wounding, while compounds like terpenes take longer to be produced (Escobar-Bravo et al. 2023).

To investigate how the presence of belowground herbivory affected the interaction between an aphid herbivore and two natural enemies, we performed a set of greenhouse and laboratory experiments with corn aphids, *Rhopalosiphum maidis;* a generalist predator, *Hippodamia convergens;* and an aphid parasitoid, *Aphidius colemani,* on maize (*Zea mays*). Specifically, we determined how presence of Western corn rootworm larvae (*Diabrotica*

virgifera virgifera; hereafter, WCR) altered aphid population growth, consumption by predators, parasitism rates, and natural enemy preference. We also determined how the presence of WCR and aphids altered aboveground maize volatile blends after 24h and 1 week of aphid herbivory. We hypothesized that parasitoids would prefer aphid hosts on plants without WCR, and would parasitize aphids on those plants at a higher rate. In contrast, we expected that the generalist lady beetle would not distinguish between aphids on either plant. Understanding these interactions will allow us to better predict how community dynamics will be affected by simultaneous above- and belowground herbivory in natural and agricultural systems.

MATERIALS & METHODS

Organisms for experiments

To investigate the effects of belowground herbivory on aboveground herbivore and natural enemy performance, we used a focal system of maize (*Zea mays*); corn aphid (*Rhopalosiphum maidis*); Western corn rootworm larvae (WCR, *Diabrotica virgifera virgifera*); the convergent lady beetle (*Hippodamia convergens*); and an aphid parasitoid (*Aphidius colemani*). Aphids were obtained from a source colony at Penn State University and were maintained on a hybrid maize line (Master's Choice 4050, King's AgriSeeds Inc.), the same line used for all experiments. Corn seeds were germinated on damp paper towel in the dark for 2-3 days prior to planting (Fig. 3.1A). Corn was grown in potting soil (Pro-Mix General Purpose, Premiertech Growers and Consumers Inc). From April-September, corn was grown in the greenhouse at approximately 24°C-27°C and a 16:8 L:D cycle in 10cm x 10 cm x 14cm pots; from October-February corn was maintained in a growth chamber at 30°C and a 16:8 L:D cycle in 10cm x 10 cm x 9 cm pots. Experiments were performed at 27°C. Aphid colonies were maintained in the laboratory at

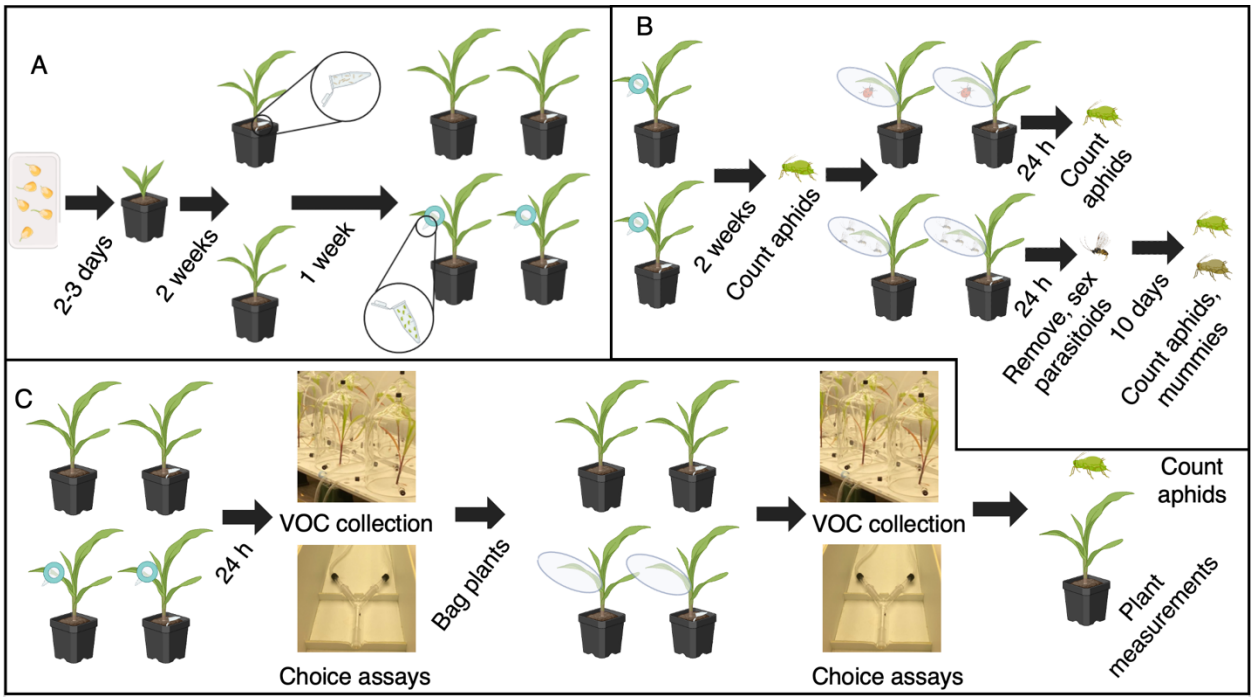

Figure 3.1. Experimental design. All plants were prepared for experiments as described in (A). Maize seeds were germinated on a damp paper towel in the dark for 2-3 days, then planted into pots. Seedlings grew for 2 weeks in the greenhouse or growth chamber prior to addition of 10 WCR neonates in treatments with belowground herbivory. After 1 week, 10 wingless adult aphids were added to plants with aboveground herbivory using a clip cage. Afterwards, plants were utilized in performance experiments (B), preference experiments (C), and volatile collection (C).

ambient temperature and light conditions for use in experiments. Western corn rootworm eggs were obtained from the USDA-ARS in Brookings, SD, and kept at 10°C until use. Two weeks prior to use in experiments, WCR eggs in soil were placed in a Petri dish inside a resealable bag with a 25 mm strip of damp paper towel. Eggs were then placed inside a Styrofoam box in growth chamber at 23°C to promote hatching. Lady beetle adults were purchased from Carolina Biological Supply Company (Burlington, NC) and stored at 1°C with access to water until 24h prior to use in experiments. At that time, adults were moved into a mesh cage (40 cm x 40 cm x 60 cm, RESTCLOUD). *Aphidius colemani* were purchased from Rincon-Vitova (Ventura, CA) insectaries as aphid mummies. Adults emerged into a mesh cage (40 cm x 40 cm x 60 cm, RESTCLOUD) where they had access to a 1:1 sugar: DI water mixture and were able to mate;

they were exposed to a corn plant with corn aphids to gain oviposition experience. Mummies were moved every 24h so that each cage contained same-age adults.

For each experiment, MC4050 maize was grown for two weeks in the greenhouse or growth chamber before adding WCR neonates to plants with belowground herbivory (Fig. 3.1A). Ten WCR neonates were placed into an 0.65 mL micro-centrifuge tube which was carefully opened and placed at the base of the stem. We observed that neonates entered the soil within an hour. Plants with and without WCR were kept in separate trays to prevent movement of larvae between pots of different treatments. A week after adding WCR, 10 adult apterous aphids were added to each plant (Fig. 3.1A). Aphids were selected under a microscope and placed into 0.65 mL micro-centrifuge tubes, which were then secured through the side of a clip cage made from silk screen printing fabric mesh and pool noodles (Haas et al. 2018; Fig. 3.1A). Although we did not count the number of WCR larvae on plants after release, we destructively sampled several plants after each experiment to ensure that WCR larvae could be found in the soil.

Performance experiment

To determine how belowground feeding by WCR larvae affected corn aphid performance and the performance of natural enemies, in June 2023, 60 maize plants were grown according to the methods described in the previous section. Thirty plants received WCR neonates, and all 60 plants received aphids (Fig. 3.1B). One week post aphid addition, clip cages were removed and adults, alates, and nymphs were counted for each plant. Leaves with aphids were bagged using 20 cm x 45 cm mesh bags for one additional week. Aphid populations were then counted a second time (Fig. 3.1B).

After the second count, plants were randomly assigned to the *H. convergens* or *A. colemani* treatment. Plants that received *H. convergens* had one adult lady beetle added to the

bagged leaf with aphids for 24h (Fig. 1B). After 24h, the lady beetle was removed and aphids were counted to get a measure of consumption. We added four adult *A. colemani* to bagged leaves in the parasitoid treatments (Fig. 3.1B). After 24h, parasitoids were removed and sexed. Mummies were allowed to develop for 10 days; mummies and emerged parasitoids were then counted.

Preference experiment

Maize (Master's Choice 4050) for choice assays and volatile collection experiments was grown in the growth chamber in January 2024. Eight-10 plants were planted each day for 5 days to ensure that they were equivalent ages when used in experiments. Plants were assigned to one of four herbivory treatments: control (no herbivory); aphids only; WCR only; or both insects. WCR neonates were added to half of the plants 1 week post-planting and plants were used in either y-tube choice assays or aboveground volatile collections 24 h after adding aphids (Fig. 3.1C). Plants were re-bagged after the experiments and returned to the growth chamber for another week, after which choice assays and volatile collections were repeated on the same plants (Fig. 3.1C).

Choice assays. To determine *A. colemani* and *H. convergens* preference for plant odors of different herbivory treatments, we used a Y-tube olfactometer (stem length = 10 cm, arm length = 6 cm, internal diameter = 1.5 cm) to conduct a series of two-choice assays in a walk-in climate chamber (27°C). Plants were placed into sealed glass cylindrical containers (height = 35 cm, internal diameter = 15 cm) with purified air passing through at a flow rate of 1 L/min. Y-tube arm position was switched after each choice; each y-tube was used for a maximum of 5 choices before cleaning with hexane and acetone, and individual plants were used for a maximum of 10 choices per species. Individuals were observed in the y-tube for 10 minutes or until it moved

>50% into one arm, which was recorded as a choice. Individuals that failed to make a choice after 10 minutes were recorded as 'no choice'. Choice assays were performed for the following plant combinations: aphids vs. control; aphids vs. WCR + aphids; and WCR vs. WCR + aphids. Twenty-four hours after aphid addition, 30-31 choices per species per comparison were performed. One week after aphid addition, 31-40 choices per species per comparison were performed.

Aboveground volatile collection. Collections were performed in a walk-in growth chamber with a 16:8 L:D cycle at 27°C for 24h. Three-week old plants (Fig. 3.1C) were placed in 9L glass chambers and a push-pull system (Analytical Research Systems) was used to collect volatiles. Purified air was pushed through collection chambers at a flow rate of 1.2 L/min. Air was pulled through traps containing adsorbent filters of 45 mg HayeSep Q (Hutchison Hayes Separation Inc., USA) at a rate of 1 L/min. After collection, traps were eluted using 150 µL dichloromethane and 5 µL of a standard containing 40 ng/µL nonyl acetate was added to all samples for quantification purposes. Samples were stored at -20°C until processing. Samples were analyzed on an Agilent 7890 GC with an Agilent HP-5MS UI column (30m x 0.25 mm x 0.25 µm) coupled with an Agilent 5795C MS with standard EI tune settings. One µL splitless injections were performed for each sample at an inlet temperature of 250°C using helium carrier gas at a flow rate of 0.7 mL/min. Following injection, the column was held at 40°C for 2 min, followed by a temperature ramp of 10°C/min to 300°C, where it was held for 4 min. Target compounds were identified in Masshunter (Table 3.1), and then those compounds were used to build a target library in the Automated Mass Spectral Deconvolution Identification System (AMDIS, version 2.73) using the NIST (National Institute of Standards and Technology (NIST), Gaithersburg, Maryland) database as a reference. Data was batch processed for the 24h and 1

week collection, then normalized to the internal standard yielding ng of each compound per sample.

Statistical analysis

All analyses were performed in R version 4.3.2 (R Core Team 2023). Aphid responses (number of adults, nymphs, total aphids, or parasitoid mummies) were analyzed using generalized linear models with a negative binomial distribution (family = 'nbinom2'), with WCR presence as a fixed effect, using the package glmmTMB. Number of aphids consumed by lady beetles was analyzed using a linear model with treatment as a fixed effect. Models were then evaluated using the 'anova' function, and pairwise comparisons were performed with the function 'emmeans'. Because single-model ANOVA methods are not available for glmmTMB models, we report comparisons of models with treatment as a fixed effect to null models. Because we performed a single round of each experiment and did not require a blocked design, we did not include random effects in the model. Y-tube assays were analyzed using a χ^2 test. Prior to analysis, headspace volatile data was square-root transformed to reduce the contribution of variation in highly prevalent compounds to dissimilarity while preserving absolute differences in emission, since aphids are known to suppress volatile emission (Schwartzberg et al. 2011), indicating that actual amount of compounds in the blend might be biologically meaningful. Headspace volatile composition by treatment was analyzed with the following functions, all from the package 'vegan' (Oksanen et al. 2022): Permutational multivariate analysis of variances (PERMANOVA) were calculated for models using the 'adonis2' function; dispersion was calculated with the 'vegdist' function (method = 'bray') followed by the 'betadisper' and 'permutest' functions; and pairwise comparisons were performed with the 'pairwise.adonis2' function. For PERMANOVA and dispersion analyses, we evaluated models with herbivory treatment as a fixed effect.

Nonmetric multidimensional scaling (NMDS) plots were created using the 'metaMDS' function; 95% confidence intervals were calculated with the 'anosim' function in the package 'vegan' (Oksanen et al. 2022). Similarity percentage analyses were performed with the 'simper' function. We also performed a partial distance-based redundancy analysis with the function 'capscale' using Bray-Curtis distance metrics using factors that were significant in the PERMANOVA. This metric was used to determine which compounds were contributing most to overall differences between different herbivory treatments. ANOVA was then used to test for effect of treatment on the capscale object.

Table 3.1. Target compounds for aboveground volatile analysis.

Compound name	**Compound name**
α-pinene	α-ylangene
Mesitylene	α-copaene
Cumene	Longifolene
β-myrcene	Caryophyllene
Limonene	*β*-elemene
β-ocimene	E-(*β*)-farnesene
Linalool	γ-cadinene
Benzyl acetate	Zonarene
Methyl salicylate	TMTT
Indole	Nonyl acetate (internal standard)

RESULTS

Performance experiment

After 2 weeks, aphid populations were 45% larger on control plants compared to plants with WCR feeding ($\chi 2 = 4.13$, df = 1, p = 0.04, Fig. 3.2A). On average, aphid populations were 194 per plant on controls (SEM = 1.14) and 134 on plants with WCR (SEM = 1.13). This difference

was largely driven by nymphs, which were higher on control plants ($\chi 2 = 4.95$, df = 1, p = 0.03, Fig. 3.2B); number of apterous adults did not differ ($\chi 2 = 0.04$, df = 1, p = 0.85; Fig. 3.2C).

Lady beetles consumed an average of 136 aphids on control plants (SEM = 19.8) compared to an average of 60 on plants with WCR herbivory (SEM = 19.8), an increase of 128% ($F_{1,28} = 7.42$, p = 0.01; Fig. 3.3A). In contrast, there was no difference in the number of mummies produced per female by *A. colemani* after 10 days ($\chi 2 = 0.11$, df = 1, p = 0.73; Fig. 3.3B).

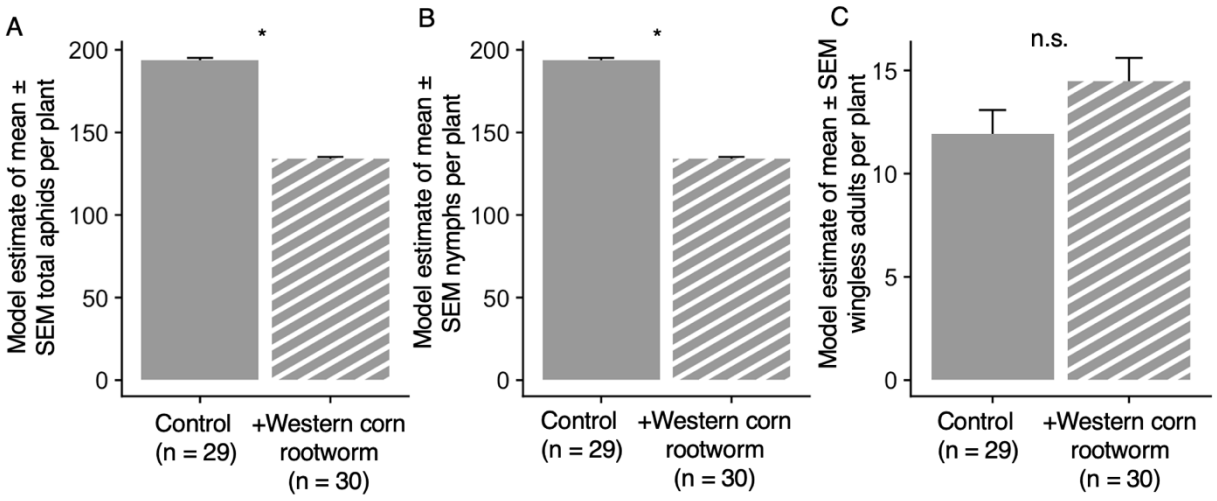

Figure 3.2. Model estimates of aphid performance. After one week of WCR feeding, aphids were added to all plants using clip cages (week 1, Fig. 3.1A); after a week, clip cages were replaced with bags (week 2, Fig. 3.1B). Model estimate of mean ± SEM aphids per plant (A). Model estimate of mean ± SEM nymphs per plant (B). Model estimate of mean ± SEM wingless adults. Asterisks indicate $p < 0.05$ difference between treatments.

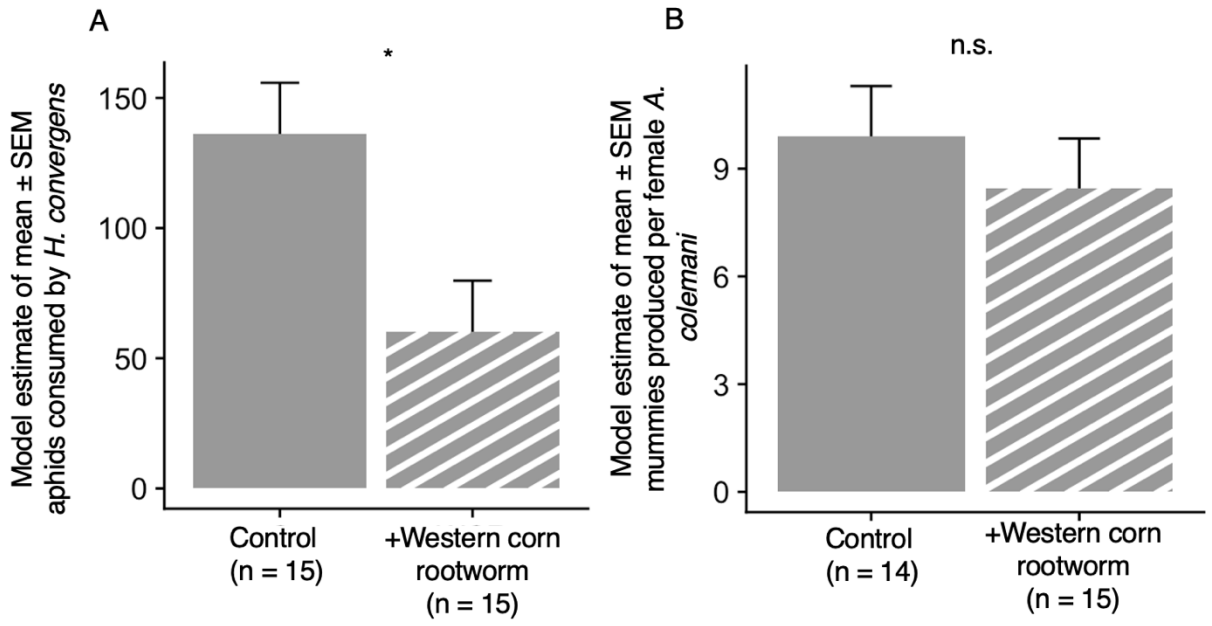

Figure 3.3. Model estimates of natural enemy performance. After two weeks of aphid feeding, one adult *H. convergens* or four adult, mated *A. colemani* were added to a bagged leaf with aphids for 24h. All natural enemies were then removed and aphids were counted (lady beetle treatment) or left for 10 days for mummy development, then counted (parasitoid treatment). Model estimate of mean ± SEM aphids consumed by *H. convergens* in 24h (A). Model estimate of mean ± SEM number of mummies per female *A. colemani*, 10 days post parasitoid exposure (B).

Preference experiment

Choice assays

After 24 h of aphid feeding, *A. colemani* chose the olfactometer arm with aphids alone twice as often as the arm with control plants in the aphids vs. control comparison ($\chi2 = 3.90$, df = 1, p = 0.048; Fig. 3.4A). *Aphidius colemani* did not distinguish between any other choices at 24h (aphids vs. WCR + aphids: $\chi2 = 0.93$, df = 1, p = 0.34; WCR vs. WCR + aphids: $\chi2 = 1.2$, df = 1, p = 0.27; Fig. 3.4A) or 1 week (aphids vs. control: $\chi2 = 0.11$, df = 1, p = 0.75; aphids vs. WCR + aphids: $\chi2 = 2.63$, df = 1, p = 0.10; WCR vs. WCR + aphids: $\chi2 = 0.9$, df = 1, p = 0.34; Fig. 3.4B). In contrast, *H. convergens* did not distinguish between any comparisons after 24 h of aphid feeding (aphids vs. control: $\chi2 = 0.03$, df = 1, p = 0.86; aphids vs. WCR + aphids: $\chi2 = 0$,

df = 1, p = 1; WCR vs. WCR + aphids: $\chi 2 = 0.86$, df = 1, p = 0.35; Fig. 3.4A). However, after 1 week, *H. convergens* chose plants with aphids and WCR over plants with WCR alone twice as frequently (WCR: 12; WCR + A: 23), though this difference was not statistically significant ($\chi 2 = 3.46$, df = 1, p = 0.06; Fig. 3.4B). No other choices were significant (aphids vs. control: $\chi 2 = 0.13$, df = 1, p = 0.72; aphids vs. WCR + aphids: $\chi 2 = 0.13$, df = 1, p = 0.72; Fig. 3.4B).

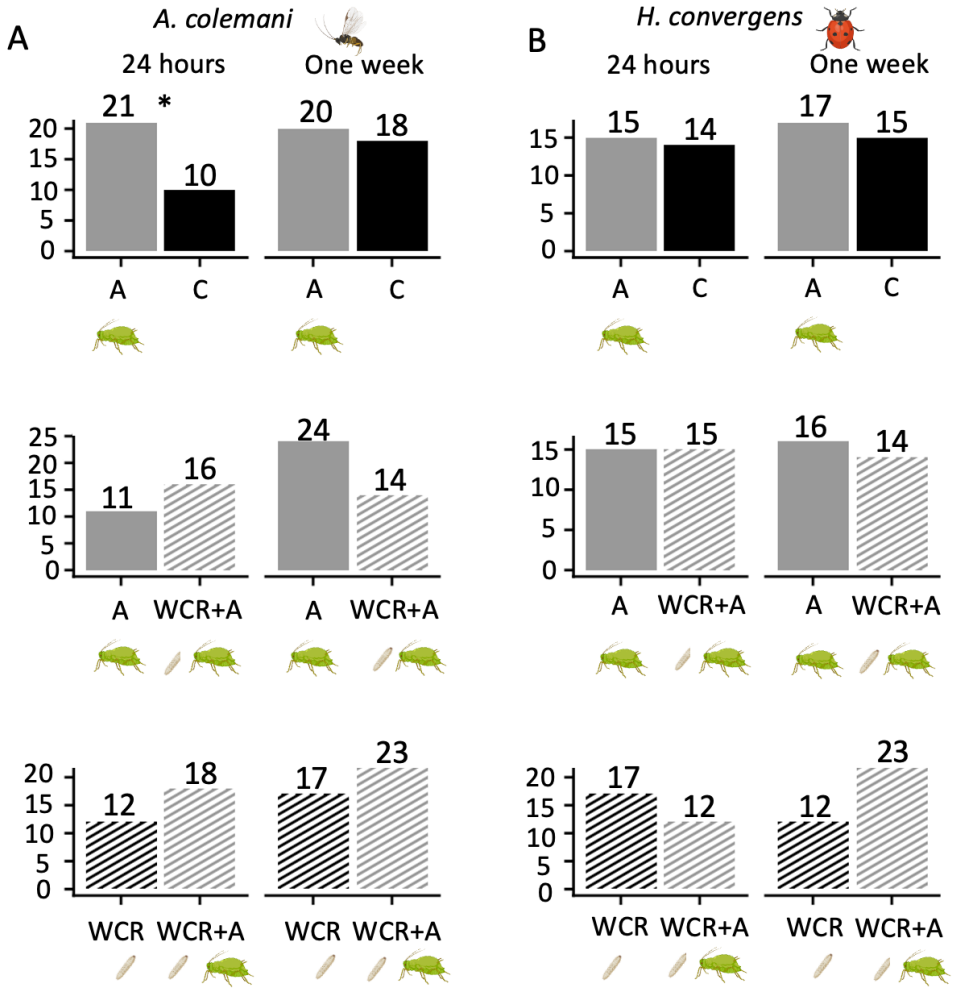

Figure 3.4. Choice assay results at 24 h and 1 week post aphid addition for *A. colemani* (A) and *H. convergens* (B). * indicates $p < 0.05$. The number of individuals making the choice is indicated within each bar. Treatments are as follows: A = aphids only; C = control; WCR = Western corn rootworm only; WCR+A = Western corn rootworm and aphids.

Aboveground volatiles

Approximately 29% of variation in aboveground volatile composition was due to herbivory treatment 24 h after the addition of aphids ($F_{3,23} = 2.71$, $p = 0.048$, $R^2 = 0.290$; Fig. 3.5A). Dispersion was not different between groups at that timepoint ($F_{3,20} = 0.31$, $p = 0.83$). Using aphids or WCR alone as a predictor did not explain differences in volatile blends ($F_{1,23} = 1.63$, $p = 0.20$, $R^2 = 0.07$ and $F_{1,23} = 1.12$, $p = 0.29$, $R^2 = 0.05$, respectively). Pairwise comparisons showed that volatile blends were distinct between control and WCR plants ($F = 7.33$, $p = 0.01$) as well as control and aphid plants ($F = 7.86$, $p = 0.02$), but similar for all comparisons at 24h ($F \leq 2.37$, $p \geq 0.14$). The aphid-control comparison yielded the highest number of compounds significantly contributing to dissimilarity (Table 3.2). Performing an ANOVA on the capscale object confirmed the significance of herbivory treatment under constrained analysis ($F_{3,30} = 1.93$, $p = 0.031$, Fig. S3.1A).

One week after adding aphids, herbivory treatment explained 25.2% of the variation in aboveground volatile composition ($F_{3,37} = 3.82$, $p = 0.004$, $R^2 = 0.252$; Fig. 3.5B). Dispersion between herbivory treatments was similar ($F_{3,34} = 2.46$, $p = 0.07$). Aphids and WCR as sole predictors explained 16% ($F_{1,37} = 7.07$, $p = 0.002$, $R^2 = 0.163$) and 8% ($F_{1,37} = 3.24$, $p = 0.045$, $R^2 = 0.083$) of VOC variation, respectively. Pairwise comparisons were significant for aphids vs. WCR at 1 week ($F = 8.58$, $p = 0.003$) and WCR vs. WCR + aphid ($F = 5.79$, $p = 0.007$) but no other pairwise comparisons were different ($F \leq 2.91$, $p \geq 0.07$). The WCR-WCR+A comparison yielded the highest number of compounds significantly contributing to dissimilarity (Table 3.2). Db-RDA confirmed the significance of herbivory treatment ($F_{3,34} = 2.57$, $p = 0.004$) and aphid presence ($F_{1,36} = 4.97$, $p = 0.001$) under constrained analysis, but not WCR alone ($F_{1,36} = 1.81$, $p = 0.106$) (Fig. S3.1C).

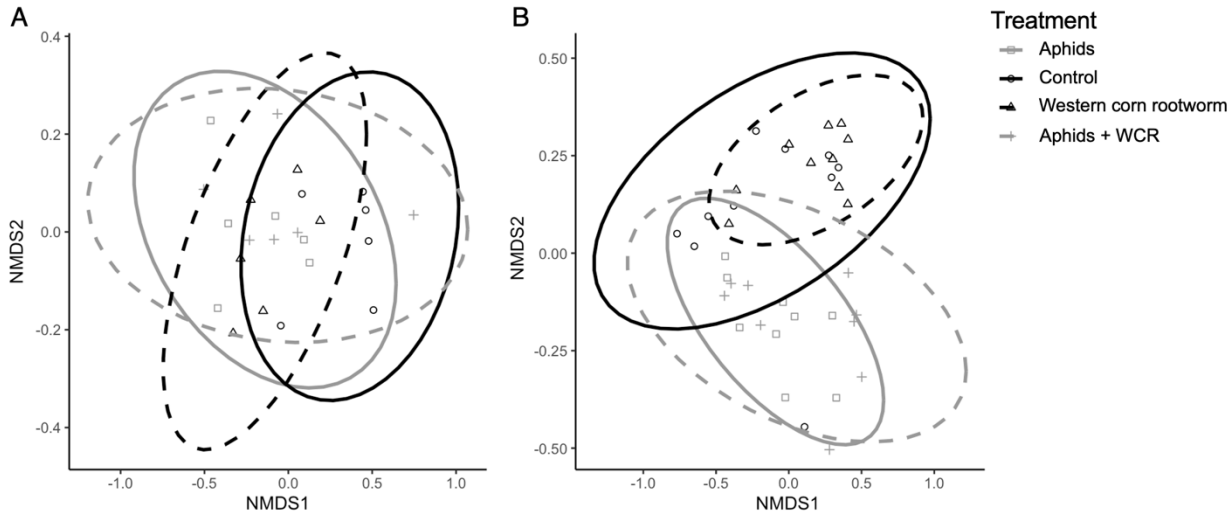

Figure 3.5. Non-metric multidimensional scaling (NMDS) plots of aboveground volatile blends of maize with the following herbivory treatments: no herbivory (control); aboveground only (aphids); belowground only (Western corn rootworm, WCR); or both (aphids + WCR) at 24 hours (A, stress = 0.06) and 1 week (B, stress = 0.06) after addition of aphids. Plants were grown for 2 weeks, at which point 10 WCR were added to belowground treatments. After one week of WCR feeding, 10 adult wingless aphids were added to plants in the aboveground treatments. Volatiles were collected for 24 h at each timepoint. N = 5-6 plants per treatment at 24 h, and 9-10 plants per treatment at 1 week. Circles represent 95% confidence intervals for each treatment.

DISCUSSION

We tested combinations of above- and belowground herbivory in maize on the preference and performance of two natural enemies: a generalist predator and an aphid parasitoid. Additionally, we examined plant volatile emission under the same herbivory treatments to link plant volatiles to predator and parasitoid preference. We predicted that parasitoids would prefer plants with aphids alone and perform better on those hosts, while lady beetles would not distinguish between the two and perform equally. We found that feeding by WCR larvae belowground reduced aphid population size aboveground after 2 weeks of feeding. Specifically, fewer nymphs were produced on plants with WCR herbivory. In contrast to our predictions, *A. colemani* produced an equal number of mummies per female between the two treatments, while lady beetles consumed more aphids on plants without WCR herbivory. However, *A. colemani* showed a preference for

Table 3.2. SIMPER analysis output for comparisons performed in y-tube choice assays with *A. colemani* and *H. convergens.* SIMPER results are presented for comparisons with accompanying y-tube choice data (control-aphids; aphids-WCR+aphids; WCR-WCR+aphids) after 24h and 1 week of aphid feeding. 'Avg' is the contribution of that compound to between-group similarity. 'sd' is the standard deviation of the contribution. 'Avg: A' and 'Avg: B' report the average abundance per group. 'p' is the permutation p-value, or the probability of an equal or larger average contribution with random permutation of the 'Treatment' factor. Compounds with $p < 0.05$ are bolded.

Comparison: A-C

	24 h					1 week					
Position	Compound	Avg	SD	Avg: A	Avg: C	p	Compound	Avg	SD	Avg: A	Avg: C	p
1	**Mesitylene**	**0.089**	**0.051**	**13.776**	**34.960**	**0.004**	Mesitylene	0.061	0.044	26.154	22.922	0.934
2	**Cumene**	**0.045**	**0.034**	**9.637**	**20.510**	**0.010**	**Benzyl acetate**	**0.041**	**0.018**	**16.557**	**8.991**	**0.001**
3	Benzyl acetate	0.023	0.017	13.759	10.350	0.834	**Methyl salicylate**	**0.038**	**0.023**	**23.086**	**14.664**	**0.021**
4	Linalool	0.022	0.016	24.993	20.040	0.548	Linalool	0.032	0.021	19.878	19.022	0.293
5	**Caryophyllene**	**0.019**	**0.012**	**5.833**	**1.400**	**0.005**	TMTT	0.029	0.020	11.051	4.976	0.194
6	**TMTT**	**0.018**	**0.008**	**8.516**	**3.940**	**0.022**	Cumene	0.028	0.021	15.812	14.681	0.994
7	**α-Ylangene**	**0.013**	**0.008**	**2.060**	**2.060**	**0.008**	β-elemene	0.010	0.009	2.781	0.353	0.119
8	Methyl salicylate	0.011	0.008	18.209	15.580	0.965	**(E)-β-farnesene**	**0.010**	**0.006**	**3.313**	**1.414**	**0.045**
9	**Limonene**	**0.011**	**0.006**	**3.004**	**5.540**	**0.009**	Caryophyllene	0.010	0.008	3.782	1.878	0.822
10	**(E)-β-farnesene**	**0.009**	**0.006**	**3.076**	**0.810**	**0.002**	β-myrcene	0.010	0.008	4.715	4.501	0.496
11	α-Copaene	0.007	0.004	4.391	2.820	0.056	α-Ylangene	0.009	0.007	2.471	2.197	0.532
12	β-elemene	0.007	0.006	1.888	0.480	0.366	Limonene	0.008	0.007	4.228	3.862	0.684
13	Zonarene	0.005	0.004	3.120	2.080	0.700	**Indole**	**0.008**	**0.006**	**2.141**	**0.342**	**0.026**
14	Indole	0.005	0.004	1.445	0.650	0.429	Zonarene	0.006	0.005	2.010	1.737	0.718
15	Longifolene	0.004	0.003	1.061	1.170	0.534	Longifolene	0.006	0.005	2.340	1.267	0.617
16	β-ocimene	0.004	0.004	0.770	0.770	0.233	α-Copaene	0.005	0.004	3.083	2.010	0.388
17	γ-cadinene	0.003	0.002	1.861	2.120	0.298	α-pinene	0.004	0.003	2.526	2.207	0.337
18	β-myrcene	0.003	0.002	6.167	5.910	0.986	β-ocimene	0.003	0.004	0.879	0.296	0.146
19	α-pinene	0.002	0.002	2.505	2.310	0.951	γ-cadinene	0.003	0.002	1.855	1.810	0.301

Table 3.2 (cont'd)

Comparison: A-WCR+A

		24 h						1 week				
Position	Compound	Avg	SD	Avg: A	Avg: WCR+A	p	Compound	Avg	SD	Avg: A	Avg: WCR+A	p
1	Mesitylene	0.059	0.051	13.776	21.642	0.619	Mesitylene	0.059	0.042	26.154	18.551	0.956
2	Cumene	0.032	0.025	9.637	12.470	0.525	Cumene	0.031	0.023	15.812	10.233	0.977
3	Benzyl acetate	0.032	0.023	13.759	18.740	0.170	Linalool	0.026	0.018	19.878	21.405	0.865
4	Linalool	0.021	0.026	24.993	23.953	0.604	TMTT	0.025	0.019	11.051	13.409	0.563
5	Methyl salicylate	0.014	0.011	18.209	17.634	0.739	Methyl Salicylate	0.025	0.018	23.086	24.958	0.860
6	Caryophyllene	0.013	0.010	5.833	4.915	0.546	Benzyl acetate	0.015	0.013	16.557	15.746	1.000
7	TMTT	0.012	0.012	8.516	7.939	0.729	Caryophyllene	0.011	0.006	3.782	4.781	0.411
8	β-elemene	0.008	0.006	1.888	2.508	0.143	β-elemene	0.009	0.007	2.781	2.965	0.221
9	Limonene	0.007	0.006	3.004	3.984	0.657	β-myrcene	0.009	0.008	4.715	4.647	0.652
10	α-Copaene	0.007	0.005	4.391	3.461	0.188	α-Ylangene	0.008	0.007	2.471	2.375	0.882
11	α-Ylangene	0.006	0.007	5.008	4.092	0.918	(E)-β-farnesene	0.008	0.007	3.313	2.795	0.585
12	Indole	0.006	0.004	1.445	1.772	0.166	Limonene	0.008	0.006	4.228	3.510	0.788
13	Zonarene	0.006	0.004	3.120	2.557	0.659	Indole	0.006	0.004	2.141	2.229	0.466
14	(E)-β-farnesene	0.006	0.005	3.076	2.690	0.602	Zonarene	0.006	0.004	2.010	2.003	0.837
15	β-myrcene	0.004	0.003	6.167	6.506	0.512	α-Copaene	0.004	0.003	3.083	3.137	0.930
16	α-pinene	0.003	0.002	2.505	2.323	0.104	Longifolene	0.004	0.003	2.340	2.089	0.973
17	Longifolene	0.003	0.003	1.061	0.723	0.797	α-pinene	0.004	0.003	2.526	2.717	0.570
18	β-ocimene	0.003	0.004	0.770	0.000	0.695	β-ocimene	0.003	0.003	0.879	0.383	0.270
19	γ-cadinene	0.002	0.003	1.861	1.720	0.827	γ-cadinene	0.003	0.002	1.855	2.040	0.767

Comparison: WCR-WCR+A

		24 h						1 week				
Position	Compound	Avg	SD	Avg: WCR	Avg: WCR+A	p	Compound	Avg	SD	Avg: WCR	Avg: WCR+A	p
1	Mesitylene	0.050	0.044	15.795	21.642	0.873	Mesitylene	0.068	0.059	11.350	18.551	0.612
2	Benzyl acetate	0.032	0.002	13.797	18.745	0.200	Cumene	0.038	0.029	7.612	10.233	0.458
3	Cumene	0.026	0.019	11.776	12.470	0.904	**Benzyl acetate**	**0.037**	**0.018**	**7.178**	**15.746**	**0.025**
4	Linalool	0.023	0.028	26.935	23.953	0.488	**Methyl salicylate**	**0.037**	**0.024**	**16.080**	**24.958**	**0.033**

Table 3.2 (cont'd)

5	Methyl salicylate	0.020	0.015	20.952	17.634	0.073	**TMTT**	**0.033**	**0.026**	**5.531**	**13.409**	**0.027**
6	TMTT	0.013	0.011	7.914	7.939	0.558	Linalool	0.027	0.016	20.152	21.405	0.805
7	Caryophyllene	0.010	0.008	5.247	4.915	0.938	**Caryophyllene**	**0.015**	**0.008**	**2.283**	**4.781**	**0.004**
8	β-elemene	0.008	0.006	0.793	2.508	0.180	**β-elemene**	**0.012**	**0.009**	**0.087**	**2.965**	**0.007**
9	Zonarene	0.007	0.006	4.079	2.557	0.114	β-myrcene	0.009	0.009	5.619	4.647	0.443
10	α-Ylangene	0.007	0.006	4.166	4.092	0.869	(E)-β-farnesene	0.009	0.004	1.730	2.795	0.132
11	α-Copaene	0.006	0.005	4.376	3.461	0.465	α-Ylangene	0.009	0.006	2.527	2.375	0.372
12	Limonene	0.006	0.006	3.056	3.984	0.844	**Indole**	**0.009**	**0.005**	**0.221**	**2.229**	**0.006**
13	β-myrcene	0.005	0.004	6.871	6.506	0.180	Limonene	0.008	0.008	2.320	3.510	0.623
14	Indole	0.005	0.004	1.307	1.772	0.564	**Longifolene**	**0.007**	**0.004**	**0.947**	**2.089**	**0.049**
15	(E)-β-farnesene	0.004	0.004	2.498	2.690	0.856	Zonarene	0.007	0.005	2.259	2.003	0.441
16	Longifolene	0.004	0.005	1.258	0.723	0.557	**α-Copaene**	**0.006**	**0.004**	**2.209**	**3.137**	**0.037**
17	α-pinene	0.003	0.002	2.575	2.323	0.149	α-pinene	0.004	0.003	1.871	2.717	0.154
18	γ-cadinene	0.003	0.003	2.106	1.720	0.580	γ-cadinene	0.003	0.002	1.642	2.040	0.534
19	β-ocimene	0.002	0.003	0.622	0.000	0.845	β-ocimene	0.002	0.003	0.238	0.383	0.826

plants with aphids alone over control plants after 24h of aphid feeding, but did not distinguish between other pairwise comparisons, while *H. convergens* did not distinguish between different herbivory treatments at 24h or 1 week in choice assays. Constrained and unconstrained analysis of aboveground volatiles demonstrated that at both timepoints the combination of above- and belowground herbivory explained more of the variation in volatile blends than either above- or belowground herbivory alone. Additionally, we demonstrated that the contribution of specific compounds to volatile blend differences between herbivory treatments varied over time, further explaining differences in parasitoid preference between the two timepoints.

While we predicted higher performance of aphid parasitoids on plants without belowground herbivory, we found that females produced an equal number of mummies on aphid colonies with and without WCR. Previous studies have found reduced performance in terms of body size and development time of emerging larvae, among other performance measures (Soler et al. 2005, Li et al. 2017), although these studies considered caterpillar hosts rather than aphids. Additionally, in mesocosms with model grassland ecosystems, *A. colemani* parasitized an equal number of *Rhopalosiphum padi* on plants with and without a belowground nematode community including plant feeders, but emerging parasitoid mortality was lower on plants with nematodes, where aphid performance was lower (Bezemer et al. 2005). We cannot rule out the possibility that this was the case for our system, since we only tracked mummy production and not the emerging generation. Another possibility is that while the total number of aphids was higher on control plants, the number of potential hosts was similar among the two treatments. *Aphidius colemani* preferentially oviposits in large aphid size classes (Lin and Ives 2003) and performs better on larger hosts (Sampaio et al. 2008). High reproductive rates seemed to be driving the difference between aphid populations on control and WCR-infested plants, since we found a

large difference in the number of nymphs but equivalent wingless adults among both treatments. Therefore, it is possible that the pool of actual (or preferred) hosts for *A. colemani* was equivalent between plants with and without belowground herbivory. Similarly, this difference in the number of nymphs could explain why *H. convergens* consumed more aphids on control plants, since coccinellids tend to display a Type II functional response, consuming more prey when prey density is high (Omkar and Kumar 2013), and require a higher number of small prey items to fulfill their nutritional needs (Xia et al. 2003, Soares et al. 2004).

The ability of natural enemies to locate plants with prey varied by timepoint and treatment comparison. Parasitoids preferred plants with aphids alone over control plants after 24h, but were unable to locate plants with aphids when belowground damage was present (Fig. 3.4). This contrasts with previous work where the presence of belowground damage in neighboring plants enhanced parasitoids' ability to locate caterpillar prey (Soler et al. 2007). This result could be partially explained by the fact that both aphids and their symbionts (Frago et al. 2017) and WCR (Hajdu et al. 2024) can reduce aboveground volatile emission. This reduction in volatile emission may explain why we did not observe a response by our generalist predator *H. convergens* in our choice assays after 24h, but did observe a response by *A. colemani* only when distinguishing between plants with aphids alone and plants with no herbivory. While previous studies have demonstrated the attraction of lady beetles to plants infested with aphids (Ninkovic et al. 2001), it is possible that our infestation rates were too low to elicit a response. Furthermore, *A. colemani* did not maintain their ability to distinguish between plants with and without aphids at the 1 week timepoint. Previous work with maize and corn leaf aphid has demonstrated that plants respond to aphid feeding hours after herbivory begins, but after 4 days of feeding, plant metabolite profiles and gene expression return to a state more similar to control plants (Tzin et

al. 2015). Other work has found almost no change in volatile emission by *R. maidis* on maize even at very high infestation rates of 400-600 aphids per plant (Turlings et al. 1998). In our study, we found an initial change in the aboveground volatile blend with the addition of aphids. However, after 1 week—well past the 4 day timepoint reported by Tzin et al.—the overall volatile blends were no longer significantly different in our pairwise comparison. Additionally, the number of compounds that were significant in the SIMPER analysis of the aphids-control comparison changed quantitatively and qualitatively, from seven compounds (mesitylene, cumene, caryophyllene, TMTT, α-ylangene, Limonene, and (E)-β-farnesene) to four (benzyl acetate, methyl salicylate, (E)-β-farnesene, and indole). Interestingly, the only overlapping compound was (E)-β-farnesene, the aphid alarm pheromone (Vandermoten et al. 2012). While this is attractive to both parasitoids (Micha and Wyss 1996, Beale et al. 2006) and lady beetles (Abassi et al. 2000), in some lady beetles it can be moderated by the presence of caryophyllene (Abassi et al. 2000), which was indeed higher than (E)-β-farnesene in our samples (Table 3.2).

Together, the preference and performance of *A. colemani* and *H. convergens* suggests that population dynamics of aphids and relative control of herbivores in a field with a patchy distribution of belowground feeding will likely differ based on the surrounding natural enemy community. Ecosystems with diverse predator assemblages can experience dampened trophic cascades (Finke and Denno 2004), and concentrating predation or parasitism on plants without belowground herbivory could enhance intraguild predation. In contrast, niche partitioning through prey size (Ye et al. 2013) or life stage (Hood et al. 2021) can reduce interspecific competition, both of which can be influenced by belowground herbivory through plant-mediated effects on herbivore body size and development time (Soler et al. 2005, Li et al. 2017). Our results suggest that soon after colonization, aphids on plants without belowground herbivory will

face the greatest risk from parasitoids such as *A. colemani*. This will likely delay the rate of increase of those populations, especially since *A. colemani*'s preference for larger life stages removes individuals with the greatest reproductive value from the population (Lin and Ives 2003). In contrast, previous work suggests that lady beetles will locate prey based on aphid density (Ives et al. 1993). This suggests that lady beetles will be attracted to control plants early in the season as their populations grow more quickly, but consume relatively equally as the season progresses based on population density. Additionally, they are likely to have a smaller influence on overall population growth rates than parasitoids which remove more reproductive individuals from the population. However, because emergent effects of multiple predators often occur that are difficult to predict from pairwise comparisons (McCoy et al. 2012), field and cage studies that can explore multiple combinations of above- and belowground herbivory along with natural enemies will help fine-tune these predictions.

In conclusion, effects of belowground herbivory by WCR cascaded up to the third trophic level where it influenced an aphid specialist parasitoid and generalist lady beetle predator in contrasting ways. These changes were mediated through effects on aphid population growth and changes to the volatile blend of plants. While most previous work has focused on the effect of belowground herbivory on parasitoids, our results demonstrate the importance of considering generalist predators as well, and suggest that better understanding the effects of natural enemies in combination could yield a more accurate understanding of population dynamics in systems with multiple herbivory.

ACKNOWLEDGEMENTS

We thank F. Mihalick and A. Hess for help in the greenhouse and lab, and N. McCartney for assistance with the GC-MS. N. Carvajal provided helpful guidance on statistical analysis. We

thank N. Constancio, J. Roedel, L. Marmolejo, R. Rantz, N. Carvajal, N. McCartney, O. Trase, F. Mihalick, A. Jennings, L. Wagner, and A. Poudel for helpful comments and feedback. This work was supported by the United States Department of Agriculture (USDA) National Institute of Food and Agriculture (NIFA) Predoctoral fellowship (project number 2021-09359) and the Michigan State University Department of Entomology Hutson Research Fellowship.

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APPENDIX 3

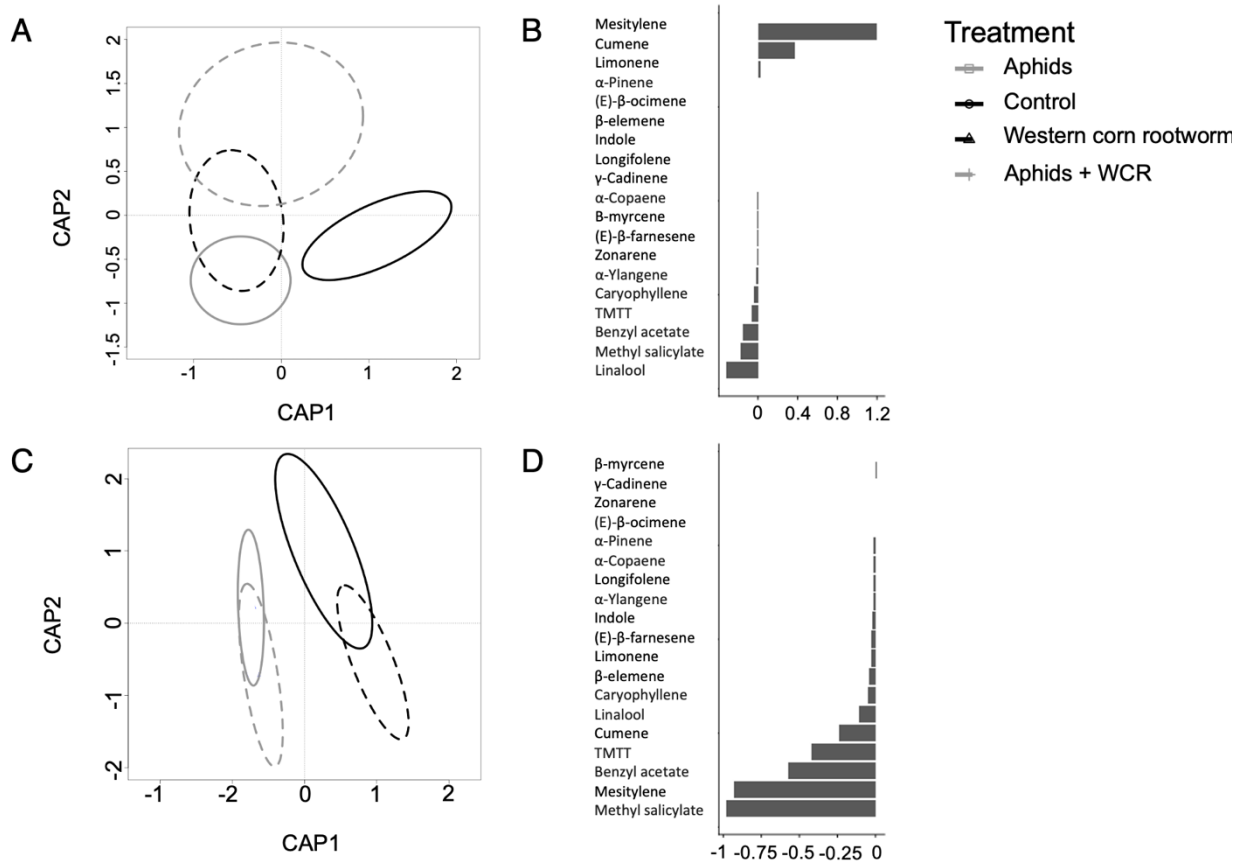

Figure S3.1. Partial distance-based redundancy analysis of aboveground volatile blends. Plants were grown for 2 weeks, at which point 10 WCR were added to belowground treatments. After one week of WCR feeding, 10 adult wingless aphids were added to plants in the aboveground treatments. Volatiles were collected for 24 h at each timepoint. N = 5-6 plants per treatment at 24 h (A), and 9-10 plants per treatment at 1 week (C). Circles represent 95% confidence intervals for each treatment. At 24h, three constrained axes explained 17%, 3%, and 2% of the variation, respectively (total = 22%; Eigenvalues: CAP1 = 0.77, CAP2 = 0.12, CAP3 = 0.11, panel A). After 1 week, three constrained axes explained 13%, 4%, and 1% of the variation, respectively (total = 18%; Eigenvalues: CAP1 = 1.17, CAP2 = 0.36, CAP3 = 0.08). Loadings (B, D) represent how emission of that compound contributes to the centroid location of a treatment. The absolute value of a compound indicates its importance for orientation on the axis while directionality indicates where on the axis treatments tend to have high emission of that compound. For example, mesitylene was highest in the control treatment after 24h (B); methyl salicylate was high both treatments with aphids (A, WCR+A) and low in the treatments without aphids (C, WCR) at one week (D), contributing to the grouping of the treatments with aphids on the left side of the CAP1 axis and those without further to the right.

CHAPTER 4:

CONCLUSIONS AND FUTURE DIRECTIONS

Multiple herbivory is common, but its outcomes vary significantly. To better understand the specific aspects of plant-mediated herbivore interactions, I performed a series of theoretical and experimental studies in my dissertation (Fig. 4.1): 1) a meta-analysis of plant-mediated interactions between insect herbivores and nematodes; 2) a set of experiments investigating the effect of constitutive plant defense on plant responses to an aboveground chewing herbivore and belowground gall nematode; and 3) a set of experiments examining the impact of below- and aboveground herbivory on the attraction and performance of two natural enemies over time. I found that foliar chewing insect growth was decreased in the presence of gall nematodes and increased in the presence of cyst nematodes, and that concurrent feeding by herbivorous nematodes and aboveground insect herbivores alters the distribution of carbon and nitrogen in the plant. Furthermore, I found that plant family and constitutive level of plant defense can alter the directionality and strength of interactions between nematodes and foliar chewing herbivores. Lastly, I determined that feeding by a belowground chewing herbivore can indirectly affect foliar aphid reproduction as well as the third trophic level through reproductive effects on aphids and aboveground plant volatiles, but that these effects change over time and affect a predator and parasitoid differently. Through these experiments, we have a greater understanding of both trends and areas that require further study. These results will be particularly relevant for crafting a pest management approach for growers who are interested in reducing pesticide use and utilizing conservation biocontrol. Furthermore, emerging crops such as hemp that have few available pesticides would be excellent systems in which to apply some of these results and next

steps. I will outline some broad takeaways and propose next steps for researchers continuing to investigate these questions.

Timing of data collection

First, it is clear that timing plays a major role in the outcomes of plant-mediated interactions. This has been highlighted previously in terms of arrival sequence, which can

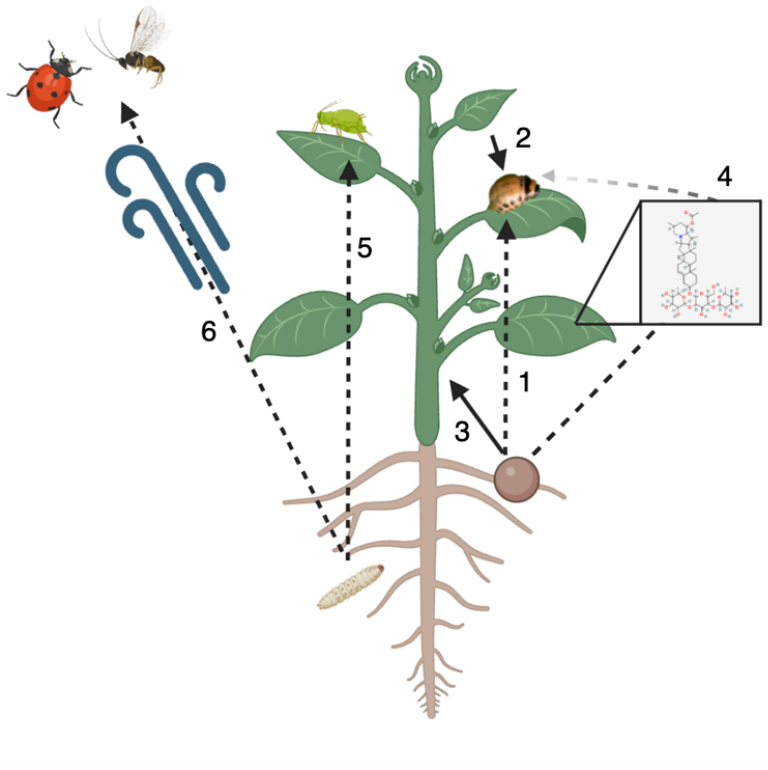

Figure 4.1. Our contributions to the understanding of plant-mediated interactions between above- and belowground herbivores. Meta-analysis results demonstrate that foliar chewing insect growth is decreased in the presence of gall nematodes and increased in the presence of cyst nematodes (1), plant-mediated interactions between aboveground herbivores and nematodes vary by plant family (2), and the concurrent presence of nematodes and aboveground insect herbivores results in altered carbon and nitrogen content in plants (3). High plant constitutive defense reduces the effect of nematodes on aboveground insect herbivores and plant defense responses (4). A belowground chewing herbivore reduced the growth rate of aphids aboveground (5), and altered the interaction between aphids and two natural enemies through differences in performance (5) and changes to the aboveground volatile blend (6).

drastically alter outcomes through effects such as priming plant defense pathways (Erb et al. 2011, Johnson et al. 2012). However, it is also important to consider how the length of an experiment and timing of data collection can alter outcomes. Many experiments are a snapshot in time and will yield different results depending on the ontogeny of all organisms involved. In my experimental work, we observed this effect when investigating both internal chemistry and plant volatiles. When plants were challenged by nematodes and later Colorado potato beetle (CPB) larvae, there was a reduction in aboveground 12-oxo-pytodienoic acid (OPDA)—the storage form of Jasmonic acid (JA)—compared to plants challenged by CPB larvae alone, indicating that the presence of Northern root knot nematode (RKN) interfered with the ability to produce OPDA. While I did not observe an effect on CPB in our experiment, I collected mass gain data at a single timepoint: 5 d post inoculation with CPB. This is less than half of the time it typically takes a CPB larva to develop from neonate to pupa. Furthermore, in a field setting, CPB are multivoltine. In Michigan, the species experiences enough degree days to complete approximately 2 generations per year (Z. Szendrei, personal communication). While plants in our experiments contained enough stored OPDA to mount an effective defense against CPB regardless of nematode presence, as evidenced by equivalent JA and JA-Ile in aboveground leaf tissue of both treatments, this reduction in OPDA could compound over a growing season and result in a much stronger effect on the second generation of CPB. In the summer generation, I would expect that CPB would perform better on susceptible plants with nematodes that had depleted their stores of OPDA and were unable to produce JA and JA-Ile.

Similarly, I observed differences in volatile blends of plants fed upon by aphids, Western corn rootworm (WCR), and both over a single week. These differences resulted in reduced preference by *Aphidius colemani* parasitoids for aphid-infested plants compared to control plants

after 1 week. Additionally, I performed again these choice tests after 3-4 weeks. However, volatile emission and composition in maize vary by phenological stage and mono- and sesquiterpene fluxes are often associated with the flowering stage (Wiß et al. 2017). Due to logistical and resource constraints, taking volatile data at only one or a few timepoints is fairly common. However, measuring the impacts of plant-mediated interactions on plants or insects only once or a few times in short succession can lead to spurious conclusions as the direction of interactions can reverse when given enough time (Johnson et al. 2013). A clear next step for future research is to attempt longer-running experiments of well-studied systems, such as cage experiments that can capture changing effects over a season, and determine whether established hypotheses hold up over time. Furthermore, working with plant breeders to screen for consistency of chemical traits among cultivars will critical for building widespread recommendations. Because chemical traits are often not the target of breeding programs, there can be significant variation between lines, including the loss of defensive abilities like release of (E)-β-caryophyllene in many maize cultivars (Hiltpold et al. 2010). Understanding these differences will help make recommendations more precise.

Identifying 'root-to-shoot' and 'shoot-to-root' effects

In both experimental studies, a hallmark of multiple herbivory was the lack of an expected change—which occurs when only a single herbivore is present—rather than an easily observable increase or decrease in a plant response. For example, when *Solanum chacoense* susceptible lines experienced herbivory by RKN and CPB simultaneously, there was a significant reduction in OPDA production aboveground compared to when plants were infested with CPB alone. However, this effect would not be identified by comparing that treatment to a non-herbivory control. Similarly, when looking at maize volatile blends under different

combinations of herbivory, volatile blends were distinct after 24h between control-aphid treatments and control-WCR treatments, but not distinct between the control-WCR+aphid treatments. This indicates that both the aphids and WCR are systemically interfering with the changes caused by a spatially separated herbivore. In some cases, 'root-to-shoot' or 'shoot-to-root' effects may look like a return to the control state rather than an additive or multiplicative increase or decrease. This is likely increasingly true as an organism's ability to interfere with a plant's chemical defense increases.

Next steps: scaling up

The majority of studies on plant-mediated interactions, current ones included, occur in the laboratory or greenhouse. My meta-analysis identified only six experiments that occurred in the field, and 43 occurred in the lab. While lab studies are critical to building a foundation of knowledge, an over-reliance on them can make it difficult to predict how interactions occur in real systems. For example, a study that found a strong negative effect of aphids and root feeding nematodes on each other in laboratory microcosms was not observed with field surveys; instead, aphid populations were higher on plants with higher root density, while cyst nematodes were higher on roots with low vitality and migratory endoparasitic nematodes were found more on plants with high root vitality and lower root density (Vandegehuchte et al. 2010). Indeed, environmental contexts can have a major impact on how extreme the effects of multiple herbivory can be. For example, plants growing in low quality soils have higher plant resistance as measured by herbivore performance compared to those grown in high quality soils (Robinson and Strauss 2018). I found that higher plant constitutive defense, one mechanism of resistance, reduced the difference in plant quality between plants with and without RKN from the perspective of CPB larvae. Through various environmental factors that reduce or exacerbate

differences in plant resistance, equivalent belowground herbivory could result in a very different landscape and performance outcome for aboveground herbivores (Fig. 4.2).

However, many constraints exist when studying belowground herbivory in an experiment in the field. Nematodes in particular are almost impossible to fully eradicate, and management often involves keeping populations at a low level rather than removing them fully (De Freitas Bueno et al. 2019). As a result, inoculating plants for long-term experiments may be difficult depending on the species. Some studies have successfully utilized pre-existing populations

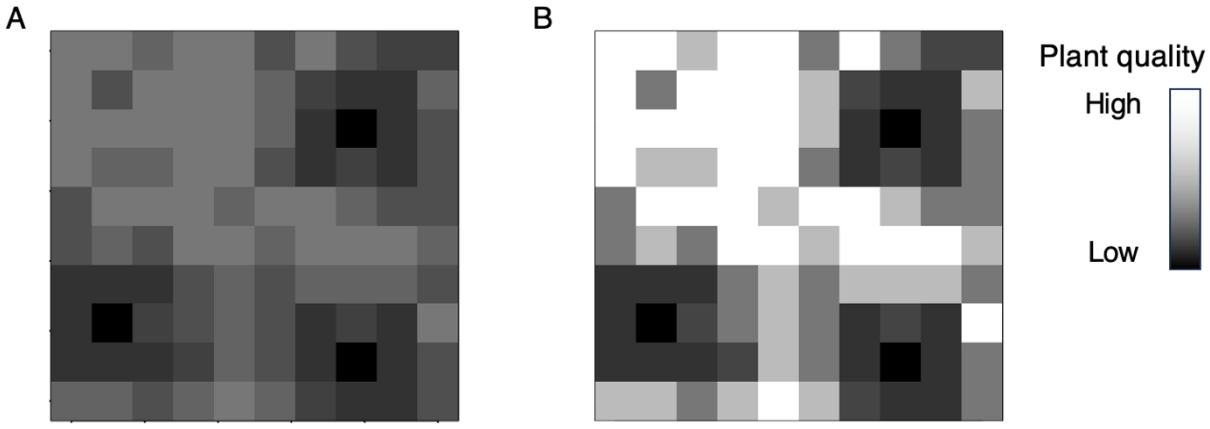

Figure 4.2. Equivalent belowground herbivory (indicated by dark patches) can result in landscapes with distinct differences in plant quality from an aboveground herbivore's perspective depending on environmental factors such as soil quality and plant constitutive defense. For example, a plant variety with high constitutive defense will result in low variation between plants with and without belowground herbivory (Fig. 4.2A) compared to a field of a plant variety with low constitutive and high inducible defense (Fig. 4.2B).

identified through surveys (Kaplan et al. 2009, Vandegehuchte et al. 2010), which is an excellent approach. Combining this approach with ecological modeling will be one way to scale up predictions of long-term and population-level effects. For example, plant parasitic nematodes and WCR larvae are patchily distributed (Goodell and Ferris 1980, Rossi et al. 1996, Park and Tollefson 2006) indicating that regardless of the direction of the plant response, their presence will likely create a patchwork of host plant quality within a field (Fig. 4.2A, B). This may

cascade up to the third trophic level through differences in insect herbivore population size and/or changes to the volatile blend (Fig. 4.3A). Previous modeling work has shown that variation in host plant quality can result in changes to the population dynamics of parasitoids and their hosts (Riolo et al. 2015); I expect a similar result will occur for fields with belowground and aboveground herbivores, with herbivore population outcomes varying based on which natural enemies are present (Fig. 4.3B). Similarly, I found a stronger effect of belowground herbivory on chewing herbivores compared to phloem-feeders in our meta-analysis, but chewing herbivores such as beetles and lepidopteran larvae can move more easily from plant to plant. Modeling may help untangle how population dynamics play out when some insects are capable of moving to escape an 'inferior' patch due to belowground herbivory, but are then more exposed to predation (Straub et al. 2014) and may feed less and experience lower survival rates (Hauri et al. 2022). Modeling outcomes will be especially useful when addressing questions of managing crop pests in agricultural systems, such as which natural enemy species will be most effective when foraging in a system with belowground herbivory (Fig. 4.3A, B).

In conclusion, a good foundation of knowledge currently exists for plant-mediated interactions between above- and belowground herbivores. Despite the difficulties that exist in scaling up these types of experiments, we can make predictions that leverage overall trends in trait-based responses combined with factors that dampen or enhance the systemic response to spatially separated herbivores. For example, the trait of herbivore feeding guild reliably induces specific defense pathways regardless of focal system. Furthermore, we can predict that this induction will be more systemically pronounced in spatially separated plant organs for plants with low constitutive defense (for example, a commercially bred potato versus a wild relative). Combining these trends with specific information about individual systems of interest could be

powerfully used to answer questions about large-scale population dynamics or effects of time through ecological modeling. By doing so, we can better harness management tools and deepen our understanding of these prevalent ecological interactions outside the lab environment.

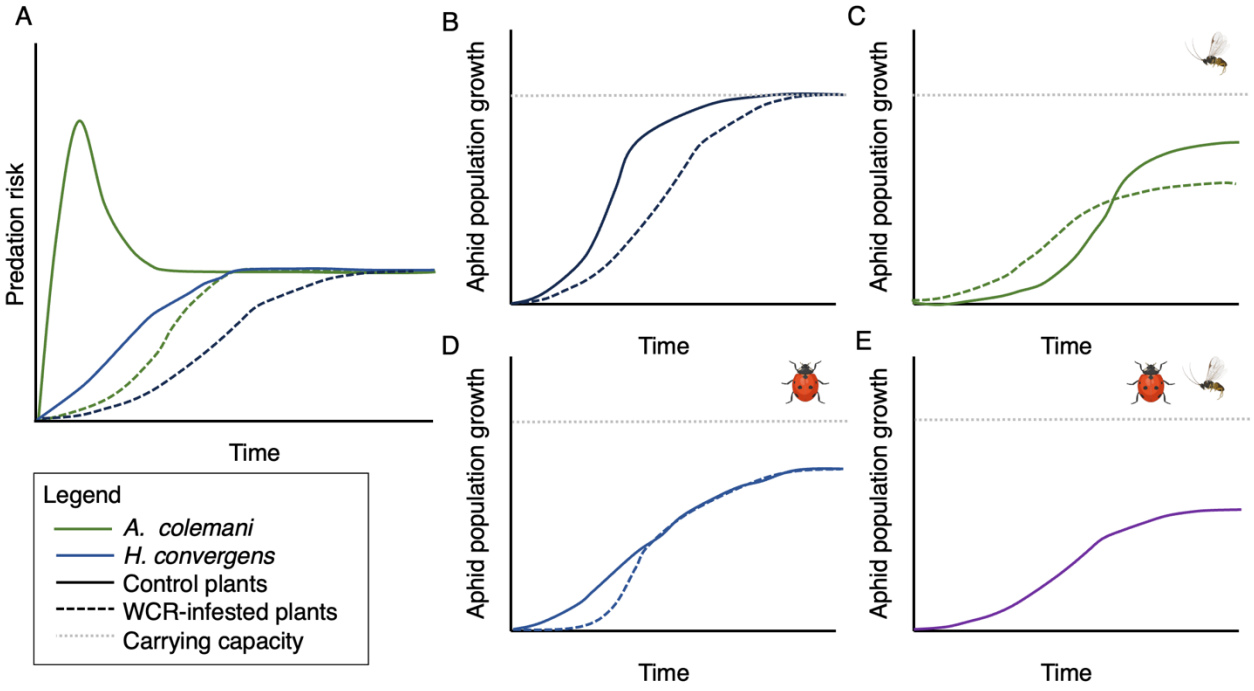

Figure 4.3. Predictions of predation risk to aphids (A) and aphid population growth (B-E) under varying belowground herbivory scenarios exposed to different types of herbivory. (A) Parasitism risk is highest for aphids on plants without belowground herbivory early on, when parasitoids show a preference for that treatment in choice assays. Additionally, at this stage, belowground infestation in other hosts may help parasitoids locate their prey on control plants (Soler et al. 2007). Lady beetles do not distinguish between volatile blends, but may be attracted to higher population density, and I predict that aphids on control plants will pass this threshold more quickly due to their higher growth rate. (B) In the absence of natural enemies, growth rate is higher on plants without belowground herbivory. (C) When *A. colemani* forage in these populations, they reduce the growth rate of colonies on their preferred control plants early on by removing reproductive individuals from the population; however, by the end of the season, preference has equalized and the growth rate on control plants can recover. (D) When exposed to lady beetles alone, I expect little difference between populations of aphids as they do not demonstrate a preference and reduced differences between populations in our performance experiment. (E) When exposed to multiple natural enemies, we expect the greatest control and relatively even distribution of aphids as *A. colemani* reduces overall reproductive capacity and *H. convergens* slows the addition of new reproductive adults to the population by consuming nymphs before they can mature.

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RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: 2024-06

Author and Title of thesis:

Author: Kayleigh Hauri
Title: Enhancing pest management of above- and belowground herbivores through plant-mediated effects

Museum(s) where deposited:
Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Specimens:

Family	Genus-Species	Life Stage	Quantity	Preservation
Braconidae	*Aphidius colemani*	adult (F)	10	pinned
Braconidae	*Aphidius colemani*	adult (M)	10	pinned
Coccinellidae	*Hippodamia convergens*	adult	10	pinned
Aphididae	*Rhopalosiphum maidis*	adult	10	alcohol
Chrysomelidae	*Leptinotarsa decemlineata*	larva	10	alcohol
Chrysomelidae	*Diaprotica virgifera virgifera*	larva	10	alcohol