

ABSTRACT

Title: INDUCIBLE PLANT RESPONSES LINKING ABOVE- AND BELOW-GROUND HERBIVORY: ECOLOGICAL SIGNIFICANCE AND UNDERLYING MECHANISMS

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Above- and below-ground organisms can indirectly affect one another via several recently-described mechanisms, one such mechanism being herbivore-induced plant responses. Because plants leaves occur above-ground and roots below-ground, systemic plant responses to foliar- and root-feeding consumers can result in reciprocal interactions between above- and below-ground herbivory.

To first address the broader theoretical context underlying plant-mediated herbivore interactions I conducted a meta-analytical review of interspecific interactions in phytophagous insects (Chapter 1). Using a data-set of 333 observations of interspecific herbivore interactions compiled from 145 independently published studies, I quantitatively assessed: (a) the overall importance of competition in the ecology of insect herbivores, and (b) whether plant-feeding insects conform to traditional competition paradigms. Despite finding frequent evidence for competition, I found very little evidence that phytophagous insects conform to theoretical predictions for interspecific competition. Notably, the strength of interactions between herbivores was largely unaffected by feeding guild, and occurred among distantly-related species that were

spatially- and temporally-separated from one another. Moreover, in most cases plants mediated these indirect interactions.

Next, I used the tobacco (*Nicotiana tabacum*) system to explore plant responses to foliar-feeding insects and root-feeding nematodes. I found that aboveground insect herbivores had limited impact on the secondary chemistry of roots, but belowground nematode herbivores strongly affected leaf chemistry (Chapter 2). However, the magnitude of leaf-root induction was also affected by vascular connectivity, with stronger induced responses among plant tissues that were more closely aligned (Chapter 3).

Last, I assessed the impact of induced responses on the performance and abundance of foliar and root herbivores using manipulative greenhouse (Chapter 4) and field studies (Chapter 5). Overall, I documented that root-feeding nematodes positively affect leaf-chewing insects by interfering with aboveground nicotine dynamics, whereas aboveground insects benefit root-feeding nematodes via alteration of source-sink dynamics.

INDUCIBLE PLANT RESPONSES LINKING ABOVE- AND BELOW-GROUND
HERBIVORY: ECOLOGICAL SIGNIFICANCE AND UNDERLYING
MECHANISMS

By

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CHAPTER 1: Interspecific interactions in phytophagous insects revisited: a quantitative assessment of competition theory

Abstract

The importance of interspecific competition is a highly controversial and unresolved issue for community ecology in general, and for phytophagous insects in particular. Recent advancements, however, in our understanding of indirect (plant- and enemy-mediated) interactions challenge the historical paradigms of competition. Thus, in the context of this rapidly developing field, I re-evaluate the evidence for interspecific competition in phytophagous insects using a meta-analysis of published studies. The analysis is specifically designed to test the assumptions underlying traditional competition theory, namely that competitive interactions are symmetrical, necessitate spatial and temporal co-occurrence, and increase in intensity as the density, phylogenetic similarity, and niche overlap of competing species increase. Despite finding frequent evidence for competition, I found very little evidence that plant-feeding insects conform to theoretical predictions for interspecific competition. Interactions were highly asymmetrical, similar in magnitude within vs. between feeding guilds (chewers vs. sap-feeders), and were unaffected by the quantity of resources removed (% defoliation). There was mixed support for the effects of phylogeny, spatial/temporal separation, and the relative strength of intra- vs. interspecific competition. Clearly, a new paradigm that accounts for indirect interactions and facilitation is required to describe how interspecific competition contributes to the organization of phytophagous insect communities, and perhaps to other plant and animal communities as well.

Introduction

Competition is routinely cited as one of the primary biotic factors that shape patterns of distribution, abundance, and diversity in ecological communities (Begon et al. 2005). The prominent status of competition in modern ecology is undoubtedly linked with views espoused by early ecologists, many of whom were strong advocates for the central role of competitive interactions between species (Darwin 1859, Grinnell 1917, Volterra 1926, Clements et al. 1929, Lotka 1932, Nicholson 1933, Gause 1934). Importantly, most biologists that are now lauded for developing and promoting traditional competition theory studied a diverse range of taxonomic groups (e.g., Clements – plants; Grinnell, Lack, MacArthur – birds; Gause – *Paramecium*; Connell – marine invertebrates; Schoener, Pianka – reptiles). Entomologists, however, were not well-represented, although several insect ecologists including Nicholson and Andrewartha provided important contributions in the debate over density-dependence. This point is noteworthy because plant-feeding insects have become focal organisms for studies on population dynamics and community diversity. Because competition is deeply ingrained in ecological theory it became widely accepted as a factor organizing insect assemblages, despite the fact that empirical evidence supporting competition was mostly derived from plant, vertebrate, and marine systems (Connell 1983, Schoener 1983, Jermy 1985, Denno et al. 1995). These circumstances set the stage for what has become a long and controversial debate over the importance of interspecific competition in structuring communities of phytophagous insects.

Initial support for the strength of competitive interactions came from observations of resource partitioning and niche segregation (see references in Denno et al. 1995). Investigations of two or more co-occurring and closely related insects revealed subtle yet distinct differences in phenology (i.e., temporal displacement) and feeding location (i.e., within-plant distribution), differences that were largely viewed as divergent strategies permitting stable coexistence (Schoener 1974, Connell 1980). Additionally, one of the best early examples of competitive exclusion came from Park's classic studies on *Tribolium* flour beetles (Park 1948). At the farthest extreme, Janzen (1973) considered all insects sharing a common host plant to be in competition with one another, regardless of niche overlap or ecological similarity: "In short, a butterfly larva that eats shoot tips can be in very intense competition with a root-eating beetle larva, even though neither is common, neither eats much of its respective plant part, neither comes in contact with each other, neither feeds on the same individual plant, and neither feeds at the same time of the year".

This view was certainly not shared by most ecologists. Davidson and Andrewartha (1948) were among the first to challenge the importance of competition based on their long-term observations of thrips on rose bushes. Inter-annual variation in the abundance of thrips was correlated with weather, and not explained by density-dependent factors such as competition. Later theoretical work by Hairston et al. (1960) argued that natural enemies regulate herbivores at low densities, green plant material (i.e., food) is plentiful, and therefore competition for resources must be rare at the 2nd trophic level. The final line of anti-competition reasoning was based on the following observations: (1) coexistence of multiple herbivores in spite of extensive niche overlap,

(2) lack of aggression among heterospecifics, or displays of “harmonious coexistence”, (3) positive density associations between competing species, and (4) plants with underutilized or vacant niches, leading to unsaturated communities (Ross 1957, Rathcke 1976, Lawton 1982, Strong 1982, Lawton 1984). As a result, interspecific competition was heavily criticized and relegated to a weak and infrequent force in phytophagous insects (Lawton and Strong 1981, Connell 1983, Schoener 1983, Lawton and Hassell 1984, Strong 1984, Strong et al. 1984, Jermy 1985).

As previously noted (Denno et al. 1995, Denno and Kaplan 2007), many of the arguments surrounding competition (both for and against) were not based on experimental field data, but rather insights that were inferred from patterns observed in nature. Beginning in the 1980s, however, a series of experimental studies emerged that provided robust support for competition involving herbivorous insects (e.g., Faeth 1986, Harrison and Karban 1986, Karban 1986, Karban 1989). Moreover, several of these examples involved temporally or spatially separated species in different feeding guilds, suggesting that traditional resource-based competition theory may severely underestimate the frequency and intensity of within trophic level interactions. This sudden increase in experimental support prompted a reversal in thinking and competition was thus reinstated as a central factor in the community ecology of phytophagous insects (Faeth 1987, 1988, Damman 1993, Denno et al. 1995). Most notably, Denno et al. (1995) found evidence for competition occurring in 76% of all pair-wise interactions reported in published studies.

The purpose of this review is to quantitatively assess whether herbivorous insects conform to the traditional paradigm of interspecific competition. Although several previous authors have addressed this issue (Damman 1993, Denno et al. 1995), I re-

evaluated the evidence for two primary reasons. First, earlier quantitative reviews employed a simple vote-counting approach, which does not allow for the rigorous testing of hypotheses offered by modern meta-analytical techniques (Rosenberg et al. 2000, Gates 2002). Second, and perhaps most importantly, the literature on interspecific interactions has changed greatly. Namely, indirect effects involving plants (i.e., induced defenses) and natural enemies (i.e., apparent competition) are far more prevalently encountered in published studies over the past ten years (Chaneton and Bonsall 2000, Ohgushi 2005, van Veen et al. 2006, Denno and Kaplan 2007). Indirect effects clearly violate many of the basic assumptions regarding interspecific competition. Indeed, some of the earliest and harshest criticism of competition came before inducible plant defenses were widely recognized: “Theoretically, induced defences open up the possibility of two species competing even though they occur on the host at different times, and/or exploit different parts of that host. There is no hard evidence that this does happen, but it is an interesting possibility” (Strong et al. 1984).

Competition theory predicts that two organisms engage in a reciprocal struggle for resources and these interactions become more intense as density, spatiotemporal co-occurrence, and ecological similarity (e.g., feeding guild, phylogenetic relatedness) increase. Therefore, this study is specifically designed to test each of these conditions using a meta-analysis of the literature on interspecific interactions among plant-feeding insects. Specifically, I calculated effect sizes to quantitatively evaluate the: (1) effect of herbivore damage (i.e., defoliation) on competitive strength, (2) magnitude of intra- vs. interspecific competition, (3) impact of phylogenetic similarity on competitive strength, (4) magnitude of within vs. between feeding guild interactions, (5) consequences of

spatial and temporal resource partitioning for competitive strength, and (6) symmetry of interactions between competing species. Overall, the results suggest that by considering indirect interactions, forces such as competition and facilitation will play an increasingly important role in structuring phytophagous insect communities, albeit in non-traditional ways.

Methods

Criteria for identifying and selecting studies

Published studies that test for interspecific competition in phytophagous insects were compiled using several different approaches. First, I surveyed the cited literature from published reviews on insect competition (Damman 1993, Denno et al. 1995), as well as reviews that dealt specifically with indirect plant- and enemy-mediated herbivore interactions (Karban and Baldwin 1997, Chaneton and Bonsall 2000, Ohgushi 2005, van Veen et al. 2006, Denno and Kaplan 2007). Next, I used the searchable database Web of Science to identify all studies that cited classic competition review papers. For example, Denno et al. (1995) provide the most comprehensive analysis available on competition involving herbivorous insects. Therefore, any recent (the past ten years) study on insect competition will have likely cited this article. Last, I performed keyword searches on Web of Science using various combinations of the following terms: interspecific competition, insect herbivore, induced plant defense, induced plant resistance, plant-mediated interactions, enemy-mediated interactions, apparent competition, interference competition, exploitative competition.

Unfortunately not all published accounts of insect herbivore competition could be used in the analysis. I specifically sought studies that compared the abundance, emigration, preference (feeding, oviposition), performance (survival, development time, relative growth rate, body size, fecundity), or feeding damage of one herbivore species in the presence and absence of a second herbivore species. Moreover, because these studies were being used for meta-analysis I only included experiments that provided means, measures of variation (standard error, standard deviation), and sample sizes for both control and treatment groups. As my interest was exclusively in herbivorous insects I did not include papers on competition involving non-insect arthropods (e.g., mites) or pollen- and nectar-feeders. I also excluded studies that used mechanical damage to simulate herbivory. Given the above criteria, my search resulted in a total of 333 observations of interspecific herbivore interactions obtained from 145 published studies (see Appendix A for a list of all studies used in meta-analysis).

Calculating and compiling effect sizes

The impact of interspecific competition on herbivore abundance, preference, performance, and/or damage was quantified by calculating an effect size (Hedges' d) for each pair-wise species interaction. The control group was designated as the competition-free environment, whereas the treatment group was exposed to competitors (except for development time which was reversed to standardize results with other performance-related variables, i.e., an increase in development time produces a negative effect size). As a result, negative effect sizes indicate that the competing species adversely affects the

focal herbivore, and the larger the effect size, the greater the magnitude of the competitive impact.

In addition to calculating effect sizes for each individual response variable (e.g., abundance, survival), I also pooled response variables to create a single composite effect size that assessed the overall consequence for each pair-wise species interaction. To do this I performed a meta-analysis on all of the response variables measured and used the resulting effect size and variance as an estimate of the total herbivore response. In other words, if an experiment quantified the impact of herbivore 1 on the development time, pupal weight, and fecundity of herbivore 2, then I conducted a meta-analysis on these three response variables to generate a single effect size for this interaction. By pooling the values to create a single common response variable, I was able to use all of the available data without pseudoreplication.

Each unique pair-wise herbivore interaction was considered an observation in the dataset. For example, if a study examined the impact of early-season caterpillar defoliation (Species 1) on two late-season caterpillars (Species 2 and 3), these were scored as two separate observations (i.e., effects of 1 on 2, and 1 on 3). Additionally, because plant traits often mediate interspecific competition (Denno and Kaplan 2007), I considered herbivore interactions as separate observations if they were tested on different plant species. For instance, if competition between two species was examined concurrently on oak and willow trees, then we scored the pair-wise interaction on oak independently of the interaction on willow.

There were only two conditions in which I violated the above criteria. First, when different authors reported on the same interaction these cases were considered separate

observations rather than pooled because of the substantial methodological differences employed between studies (this circumstance was exceedingly rare in the dataset). If the same author, however, reported on the same interaction across multiple publications, then the response was pooled and considered a single observation. The second condition for violating the above criteria involved differences in experimental design. If competition was assessed under fundamentally different ecological circumstances then I separated rather than pooled the observations. Typically this involved aspects of temporal or spatial segregation. For example, several studies compared the competitive impact of two species reared contemporaneously vs. when they were raised in spatial or temporal isolation from one another (i.e., prior infestation and subsequent removal of one of the two species). Because these experiments represent drastically different competitive conditions, they were considered unique observations in the dataset. However, I pooled responses into a single observation in all other circumstances (e.g., field and greenhouse assessments, competition under varying nutrient/water regimes, etc.).

To test if taxonomic affiliation or herbivore feeding guild affected the strength of competitive interactions (=effect size), I classified each herbivore species by taxonomy (order, family, genus, species) and feeding guild (chewer or sap-feeder). Additionally, the extent of plant damage was noted if such information was provided by the study. This value was calculated (and typically reported) as the percentage of leaf area removed from the plant, rather than absolute quantity of tissue damaged. Thus, the impact of plant damage on competitive strength could only be determined for cases where the competitor was a chewing insect. I did not attempt to quantify herbivory for sap-feeding insects because their feeding damage is often unapparent and, unlike defoliation, herbivore

density is difficult to standardize across taxonomic groups. Last, I assessed whether the pair-wise interaction involved herbivores that were feeding at the same time and place (direct interactions), compared with cases where the herbivores were spatially and/or temporally separated from one another (indirect interactions).

In addition to calculating effect sizes for interspecific competitors, I also scored intraspecific interactions for the sub-set of studies that assessed both inter- and intraspecific competition. However, I did not evaluate all published accounts of intraspecific competition; it was only assessed when included as part of a study on interspecific competition. This allowed for a direct comparison of the relative strengths of competition occurring within vs. between species.

For studies that used reciprocal designs (i.e., those that measured the impact of Species 1 on 2, and also the impact of Species 2 on 1), I quantified the symmetry of this relationship. Reciprocal interactions were paired and the larger of the two effect sizes was used to define the dominant competitor, whereas the smaller effect size defined the inferior competitor. If interactions were symmetrical then these two effect sizes should be equal in magnitude. Oftentimes reciprocal interactions were tested using temporally-separated species whereby plants were previously damaged by Species 1 and subsequently offered to Species 2, and vice versa. Consequently, the relative symmetry of interspecific competition was assessed for the full dataset, as well as independently for both direct and indirect interactions.

Statistical analysis

The meta-analytical program *MetaWin 2.0* was used to calculate effect sizes and conduct the overall analysis (Rosenberg et al. 2000). Sample size dictated which response variables were statistically analyzed and thus which data are presented. For categorical models, I only analyzed response variables that included >5 observations per group. Similarly, for continuous models (regression) I used response variables with >10 observations. As a result, the analyses are robust to spurious conclusions that can be generated from small sample size (Gates 2002).

Mixed-effects models were used throughout, as advocated in recent publications (Gurevitch and Hedges 1999, Rosenberg et al. 2000). Resampling tests (999 iterations) were performed to generate cumulative effect sizes with 95% bootstrap confidence intervals. This approach is suggested to account for the violation of basic distributional assumptions (e.g., normality) that typically occurs with meta-analytical data (Adams et al. 1997, Gurevitch and Hedges 1999). To account for the problem of multiple comparisons that inflate the probability of committing a type-I error, I used the more conservative $P = 0.01$ value as the cut-off for statistical significance (Gates 2002).

Initially, I analyzed the entire dataset using a model with no data structure to determine the grand mean effect size and confidence intervals for each individual response variable. This allows for an overall assessment of the strength of interspecific interactions and potential differences in the magnitude of competition across response variables (i.e., Does competition have a stronger impact on fecundity compared with abundance?).

Next, I used continuous and categorical models to test fundamental assumptions underlying competition theory. First, a weighted least squares regression was used to

assess the impact of resource removal (% defoliation) by Species 1 on the composite effect size for Species 2. Categorical models were used in all subsequent analyses to compare effect sizes between groups of predictor variables. Specifically, I used the following pre-planned contrasts: (1) intra- vs. interspecific competition, (2) direct interactions (i.e., no separation) vs. interactions between spatially and/or temporally separated species, (3) closely- vs. distantly-related species, (4) within vs. between feeding guilds, and (5) dominant vs. inferior competitors (i.e., symmetry) (see figure legends for additional details on between group contrasts). For each group a mean effect size (d_+) was calculated and reported with 95% bootstrap confidence intervals; effects are considered statistically significant if confidence intervals do not bracket zero. Between-group heterogeneity (Q_B) was tested against a χ^2 -distribution to determine if significant differences exist among groups of predictor variables.

To address the “file-drawer problem” whereby non-significant effects are less-likely to be published, I used Rosenthal’s Method ($\alpha = 0.05$) to calculate fail-safe values for each variable in the dataset (Rosenberg et al. 2000). This estimates the number of non-significant observations that are needed to change the outcome from significant to non-significant. Additionally, I created and visually assessed weighted histograms, funnel plots, and normal quantile plots for each variable to identify potential publication bias and abnormalities in the data structure (Rosenberg et al. 2000).

I found little evidence for publication bias in the dataset. Fail-safe values tended to be large, indicating that the analyses are robust to the file-drawer effect (fail-safe = 235, 82, 94, 359, 256, 2382, 2184, 361, respectively, for development time, relative growth rate, body size, survival, oviposition, fecundity, abundance, plant damage). Similarly,

weighted histograms, funnel plots, and normal quantile plots did not indicate abnormalities in the data structure.

Results

Evidence for interspecific herbivore interactions was detected in 73% (243 of 333) of all interactions in the dataset. The majority of observations (62%; 205 of 333 interactions) documented competition, whereas fewer cases provided evidence for facilitation (11%; 38 of 333). The mean effect size for each response variable was significantly less than zero in the overall analysis (none of the confidence intervals overlap zero), but the magnitude of the effects varied greatly (Figure 1.1). An effect size of 0.2 is considered small, 0.5 moderate, and 0.8 large (Cohen 1988). Therefore, competition had weak effects on body size (-0.2448) and plant damage (-0.2790), moderate effects on abundance (-0.5398), survival (-0.4502), development time (-0.6432), and relative growth rate (-0.4579), and large effects on fecundity (-1.0393) and oviposition preference (-0.8816).

Defoliation intensity by leaf-chewing insects had no impact on the effect size of heterospecific competitors ($Q_{\text{reg}} = 0.43$, $P = 0.5102$, $n = 64$; Figure 1.2). The mean effect size was significantly less than zero ($d_{++} = -0.4081$), indicating that leaf damage adversely affected subsequent herbivores. However, the slope of the regression did not differ from zero, suggesting that the quantity of tissue removed did not affect the strength of interspecific competition.

Both intra- and interspecific competition adversely affected phytophagous insects (Figure 1.3). There was little evidence, however, that the magnitude of interspecific

competition differed from that of intraspecific competition (Figure 1.3), including effects on survival ($Q_B = 0.93$, $P = 0.3339$), body size ($Q_B = 1.49$, $P = 0.2224$), oviposition preference ($Q_B = 0.14$, $P = 0.7030$), emigration ($Q_B = 2.62$, $P = 0.1056$), growth rate ($Q_B = 1.96$, $P = 0.1619$), fecundity ($Q_B = 0.47$, $P = 0.4940$), and the composite response ($Q_B = 3.12$, $P = 0.0774$). Although for every response variable there was a trend for the conspecific effect size to be greater than the associated heterospecific interaction, none of these differences was significant.

The phylogenetic similarity between competing species was a poor predictor of the strength of interactions among chewing insects (Figure 1.4A; $Q_B = 4.18$, $P = 0.2417$). Competition was weaker between congeners than between more distantly-related chewers. This finding is contrary to the expectation of traditional competition theory. Sap-feeding insects, however, provided support for theoretical expectations; phylogenetic relationship influenced the strength of their interactions (Figure 1.4B; $Q_B = 41.34$, $P < 0.0001$). This effect was driven by congeners, whose effect size was ~4x greater than more distantly-related species.

Generally, there was no difference in the strength of interactions occurring within vs. between feeding guilds (Figure 1.5). This was true when assessed for abundance ($Q_B = 0.04$, $P = 0.8477$), development time ($Q_B = 0.82$, $P = 0.3652$), oviposition preference ($Q_B = 2.19$, $P = 0.1393$), plant damage ($Q_B = 0.02$, $P = 0.8771$), body size ($Q_B = 0.01$, $P = 0.9424$), survival ($Q_B = 0.18$, $P = 0.6742$), and the composite response ($Q_B = 0.74$, $P = 0.3885$). Relative growth rate ($Q_B = 21.82$, $P < 0.0001$) was affected by within but not between feeding guild interactions. However, I do not consider this to be particularly

strong evidence because only six observations were available for estimating the magnitude of inter-guild effects on RGR.

Spatiotemporal co-occurrence had mixed effects on the strength of interspecific interactions (Figure 1.6). For certain response variables there was no effect of separation [growth rate ($Q_B = 1.63$, $P = 0.6526$), survival ($Q_B = 7.41$, $P = 0.0600$)], whereas for other response variables there was [body size ($Q_B = 21.09$, $P = 0.0001$), fecundity ($Q_B = 19.70$, $P = 0.0002$), abundance ($Q_B = 12.52$, $P = 0.0058$), composite response ($Q_B = 25.09$, $P < 0.0001$)]. No consistent patterns emerged from this analysis. In some instances (e.g., abundance) direct interactions resulted in strong competitive effects, whereas spatiotemporal separation dampened this effect, which is consistent with theoretical expectations. In other cases (e.g., growth rate) the two effects were similar in magnitude, and for two response variables (body size and survival) spatiotemporal separation resulted in stronger competitive interactions than in direct encounters.

For the majority of response variables measured, competition was highly asymmetric (Figure 1.7), including effects on survival ($Q_B = 8.76$, $P = 0.0031$), fecundity ($Q_B = 7.56$, $P = 0.0060$), and abundance ($Q_B = 9.78$, $P = 0.0018$). Body size ($Q_B = 6.04$, $P = 0.0140$) and growth rate ($Q_B = 2.04$, $P = 0.1527$) were not significant, perhaps due to smaller sample size, but demonstrated strong trends in the same direction as the other response variables. Similarly, the composite response demonstrated a high degree of asymmetry (Figure 1.8A; $Q_B = 28.42$, $P < 0.0001$), with the effect of the dominant competitor being ~4x greater than the reciprocal effect from the inferior competitor. Although both direct interactions (Figure 1.8B; $Q_B = 11.35$, $P = 0.0007$) and indirect

interactions (Figure 1.8C; $Q_B = 18.26$, $P < 0.0001$) were asymmetrical, the magnitude of the asymmetry was considerably greater for indirect effects.

Discussion

The overwhelming evidence from this meta-analytical evaluation of interspecific interactions suggests that competition is indeed an important factor influencing the performance and fitness of phytophagous insects. I detected evidence for competition or facilitation occurring in 73% of all pair-wise interactions, which corresponds with frequencies documented in previous reviews on insect herbivore competition (Damman 1993, Denno et al. 1995). Moreover, the strength of interactions documented in this study is comparable to other meta-analyses conducted on similar ecological forces (e.g., predation and competition). Halaj and Wise (2001), for example, found that predators reduced the abundance of terrestrial arthropod herbivores ($d = 0.77$). Similarly, Gurevitch et al. (1992) reported that competition affected herbivores as a whole ($d = 1.14$), but had very weak effects on terrestrial arthropod herbivores ($d = -0.04$). This earlier assessment of insect herbivore competition by Gurevitch and colleagues, however, was conducted before the recent emergence of studies on indirect interactions and thus suffers from small sample size.

Although the overall effect sizes in the analyses changed depending on the response variable measured (Figure 1.1), the magnitude of these effects are within the range documented in the above-cited meta-analyses of predation and competition involving other trophic and taxonomic groups. In fact, I suspect that the effect sizes reported in our study underestimate the ecological significance of competition because of

the pervasive impact of facilitation and asymmetry in phytophagous insect communities. Positive interactions (i.e., facilitation) are considered to be common in terrestrial insects (Denno and Kaplan 2007, Marquis and Lill 2007), and are evident in the dataset (Figure 1.1; all response variables include multiple effect sizes >0). Asymmetrical interactions are far more common than symmetrical ones (see ‘Symmetry’ section below), and the overall impact of the dominant competitor was quite large ($d = -1.1$) (see Cohen 1988). The consequence of including facilitation and asymmetry in this assessment is that average effect sizes are diluted throughout and thus provide a conservative estimate of the impact of competition.

Despite empirical support for the overall strength of competition in phytophagous insects, I found very little correspondence with traditional paradigms. Competition is not correlated with levels of defoliation, occurs among distantly-related species in different feeding guilds, is not generally dampened by spatial and temporal resource partitioning, and is highly asymmetric.

(1) Quantity of resources consumed

Exploitative competition occurs when one species consumes and thus reduces the quantity of resources available to competitors (Begon et al. 2005). Therefore, the impact of resource removal on potentially competing species should be correlated with the quantity of resources removed (i.e., intense competition when resource removal is high). In fact, the observation that herbivorous insects typically consume a small fraction of potentially available plant foliage has long been used to justify the stance that competition is rare among insects (Hairston et al. 1960). However, I found no

relationship between defoliation (%) and the strength of interspecific competition (Figure 1.2), supporting the notion that even small amounts of damage can induce systemic defenses with important ecological effects (Denno & Kaplan 2007).

This result is not simply an artifact of combining multiple dissimilar plant systems. Induced resistance to beet armyworm caterpillars (*Spodoptera exigua*) on tomato, for example, occurs at very low levels of defoliation, and the magnitude of this effect does not change as total leaf area removal varies from <1% to 10% (Stout and Duffey 1996; but see Table 4.5 in Karban & Baldwin 1997 for cases where damage intensity is correlated with induced resistance). Other studies have also documented strong interspecific effects at defoliation levels where the quantity of available leaf tissue is clearly not limiting for herbivores (e.g., Harrison and Karban 1986, Agrawal 1999, 2000, Wise and Weinberg 2002, Van Zandt and Agrawal 2004a, 2004b, Viswanathan et al. 2005).

An additional factor that confounds the relationship between resource removal and competition is interspecific facilitation. High levels of defoliation can elicit a *positive* response in subsequent herbivores by weakening plant defense systems (e.g., resin flow in conifers), or stimulating a re-flush of young, nitrogen-rich leaves (Rockwood 1974, Potter and Redmond 1989, Rieske and Raffa 1998, Wallin and Raffa 2001).

Overall, these results make it very difficult to predict competitive outcomes based purely on the amount of leaf tissue consumed, and consequently herbivorous insects may not fit the traditional paradigm for exploitative interactions. Thus, in light of inducible plant defenses, the theoretical basis underlying the argument that “herbivores are seldom

food-limited...and therefore are not likely to compete for common resources” (Hairston et al. 1960) appears seriously flawed.

(2) Phylogenetic similarity

The hypothesis that closely-related species compete more intensely than distantly-related ones has long been considered a truism in ecology. Even Darwin predicted that “the most closely-allied forms – varieties of the same species, and species of the same genus or related genera – which, from having nearly the same structure, constitution and habits, generally come into the severest competition with each other” (Darwin 1859). Therefore, the relationship between phylogenetic similarity and interaction strength is a fundamental tenet deeply embedded in competition theory. I tested the impact of phylogenetic similarity at two different levels: (1) intra- vs. interspecific competition, and (2) taxonomic relatedness among interspecific competitors.

Previous competition reviews provide mixed support for competition occurring within vs. between species (Connell 1983, Gurevitch et al. 1992, Denno et al. 1995). Although I found trends in all of the response variables for conspecifics to compete more intensely than heterospecifics, none of these differences were statistically significant indicating that intra- and interspecific competitive effects are comparable in magnitude (Figure 1.3). Similarly, taxonomic relatedness had mixed effects on interspecific competitors. Chewing insects do not conform to theoretical expectations (Figure 1.4A), whereas sap-feeders provide some support with congeners competing more intensely than distantly-related species pairs (Figure 1.4B). As a whole, the evidence for phylogenetic similarity promoting competitive interactions is relatively weak. Rather, our findings

support the more recent view that competition frequently occurs among divergent taxa (Hochberg and Lawton 1990), including distantly-related herbivorous insects (Van Zandt and Agrawal 2004b, Denno and Kaplan 2007).

I suspect that the lack of correspondence with traditional paradigms in this instance stems from the fact that, historically, competition theory has emphasized direct interactions, whereas many documented cases of competition in phytophagous insects are indirect and mediated by plants and/or natural enemies (Damman et al. 1993). As a result, the nature of the indirect effect should be more important than the degree of phylogenetic similarity. For instance, many herbivore-induced plant allelochemicals (e.g., nicotine) have broad-spectrum efficacy and thus do not discriminate among taxa (Karban and Baldwin 1997). Consequently, one herbivore can induce plant defenses that impact other herbivores sharing the host-plant, independent of their phylogenetic relationship. Plant damage by sap-feeding whiteflies, for instance, elicits elevated expression of defensive proteins that negatively affect leaf-chewing caterpillars (Inbar et al. 1999). In a similar manner, enemy-mediated interactions (i.e., apparent competition) can link distantly-related herbivores when the shared enemy is a polyphagous predator that indiscriminately attacks prey. Cotton aphids, for example, strongly attract aggressive fire ants onto plant foliage and these ants subsequently attack co-occurring herbivores, including caterpillars and other distantly-related taxonomic groups of insects (Kaplan and Eubanks 2005).

(3) Intra- vs. inter-guild interactions

Because herbivores in the same feeding guild consume plants in a similar manner they are considered more likely to compete with one another than herbivores in

discrepant feeding guilds. However, early experimental field studies on insect competition contradict this hypothesis (Faeth 1986, Karban 1986, 1989). Unlike Denno et al. (1995) who found support for guild differences diminishing competition, I found no evidence that the strength of intra-guild interactions (chewer/chewer, sap-feeder/sap-feeder) differs from that of inter-guild interactions (chewer/sap-feeder) (Figure 1.5). Because I grouped herbivores according to major feeding guilds (e.g., chewer or sap-feeder), rather than dividing them into sub-guilds (e.g., phloem-feeder, leaf-miner, gall-former, etc.), the results might underestimate the potential for guild differentiation to mediate competitive strength. For example, one might expect that concealed feeders (e.g., leaf-miners) confined to very specific resources compete more intensely than free-living chewers (Denno et al. 1995). Unfortunately, due to lack of adequate replication, the analysis of sub-guild interactions could not be performed. Nonetheless, inter-guild interactions are now well-documented in a variety of systems (Karbon 1986, 1989, Inbar et al. 1999, Kessler and Baldwin 2004, Van Zandt and Agrawal 2004b, Lynch et al. 2006, Kaplan et al. 2007). Future studies that quantify the mechanistic underpinnings of plant responses to herbivores (especially sap-feeding insects), including secondary metabolite expression and source-sink dynamics, will greatly contribute to our understanding of when and why inter-guild interactions occur (Karbon and Baldwin 1997, Denno and Kaplan 2007).

(4) Spatial and temporal resource partitioning

When two herbivores that share a common host-plant co-occur in time and space, competition is predicted to be intense. Partitioning the resource, however, by emerging

and feeding at different times of the growing season and/or utilizing different parts of the plant is thought to differentiate niches and thus diminish competition (Schoener 1974, Connell 1980). Based on their earlier review of the literature, Denno et al. (1995) concluded that resource partitioning dampens, but does not preclude competition between temporally and spatially separated species. My data tend to support this view, although the results were mixed and depended on which response variables were measured (Figure 1.6). The ecological significance of competition between spatially and temporally separated species will ultimately be determined by the spatial extent and temporal persistence of indirect plant- and enemy-mediated effects.

Herbivore-induced plant defenses are generally considered to be strongest at the site of attack soon after damage occurs, and these effects wane with time and space (Karban and Baldwin 1997). Plant induction, however, can nonetheless link spatially and temporally separated species. For example, the phytophagous mirid bug, *Tupiocoris notatus*, induces resistance to hornworm caterpillars, *Manduca quinquemaculata*, on wild tobacco, and this effect is as strong or stronger when caterpillars are fed systemic, undamaged leaves compared with locally damaged leaves (Kessler and Baldwin 2004). Similar results were found for the impact of gypsy moth, *Lymantria dispar*, defoliation on swallowtail caterpillars, *Papilio canadensis* (Redman and Scriber 2000). Induced defenses can also persist long after damage to the plant occurs. Several studies have found poor correlations between time from induction and the strength of induced resistance (Gibberd et al. 1988, Stout and Duffey 1996); in these cases the effects of induced resistance persist for at least 2-3 weeks after damage. Similarly, inter-year carryover effects from outbreak defoliators (e.g., DIR – delayed induced resistance) allow

for plants to mediate interactions between herbivores across different growing seasons (Kaitaniemi et al. 1998, 1999).

Natural enemies can also extend herbivore competition across time and space, although their spatiotemporal effects are likely to differ from plants. Because many natural enemies actively search for prey, I suspect that their temporal persistence will be less than induced plant defenses. In other words, the impact of early-season herbivores on late-season herbivores via natural enemies may be weak because predators and parasitoids will likely emigrate in search of alternative food after their focal prey disappear. Unlike induced defenses, however, natural enemies can mediate competition between herbivores on different plants, and therefore their spatial reach is far greater than induced defenses (but see Karban et al. 2006; Baldwin et al. 2006 for evidence of inter-plant communication via damage-induced volatiles). The invasion of soybean aphids (*Aphis glycines*) in the Midwestern United States, for instance, has increased the abundance of predaceous coccinellid beetles that migrate into alfalfa fields and severely reduce pea aphid (*Acyrtosiphon pisum*) densities (Harvey 2007). As a result, soybean aphids strongly “compete” with pea aphids, even though neither feeds on the same host-plant and their respective hosts occur in different agricultural fields that are separated by great distances.

(5) Symmetry

Historically, interspecific competition is thought to entail a reciprocal struggle for resources, although most prior reviews do not support this prediction (Lawton and Hassell 1981, Connell 1983, Schoener 1983, Strong et al. 1984, Denno et al. 1995, Denno

and Kaplan 2007). In fact, the lack of symmetry in interspecific interactions is one of the few generalities that emerge and appear to remain consistent across trophic levels and taxonomic groups. Our data strongly support this conclusion for phytophagous insects (Figures 1.7 and 1.8A). Moreover, by grouping effect sizes according to the dominant and inferior competitors for each interspecific pair I could quantify the degree of reciprocal effects and thus assess the symmetry of interactions. In this case, the impact of the dominant competitor was 4x greater than the reciprocal effect of the inferior species. Importantly, the inferior competitor had a significant, albeit weak, reciprocal effect in direct interactions (Figure 1.8B), but its impact was non-significant when assessing indirect (mostly plant-mediated) interactions (Figure 1.8C). This pattern suggests that asymmetry is far more pronounced with indirect effects and offers strong support for the view that indirect interactions violate the traditional dogma of competition theory.

The mechanisms underlying competitive dominance, and thus asymmetrical interactions, are diverse (e.g., aggregation, dispersal, tolerance of allelochemistry; see Denno and Kaplan 2007). For instance, early-season exploitation of host-plants creates a condition in which initial colonizers affect subsequent herbivores, but reciprocal effects of late-season feeders on early-season herbivores are unlikely. Thus, inherent differences in the phenology of insects on plants in seasonal environments promote asymmetry.

In other instances, however, the mechanisms generating asymmetry are far more idiosyncratic and unpredictable. For example, two phytophagous beetles (*Psylliodes affinis* and *Plagiometriona clavata*) commonly attack the nightshade *Solanum dulcamara*; in this system, *P. affinis* has strong plant-mediated effects on *P. clavata*, but *P. clavata* has no reciprocal impact on *P. affinis* (Viswanathan et al. 2005). In this

example, both herbivores are beetles in the family Chrysomelidae and experiments controlled for the quantity of leaf tissue damaged. Therefore, the asymmetry in this interaction is likely due to interspecific differences in feeding patterns, salivary constituents, or other undetermined factors. Similarly, gall-forming herbivores can dominate interactions with other sap-feeding insects by inducing stronger metabolic sinks as a consequence of positioning their gall basal to potentially competing species on the same leaf (Inbar et al. 1995).

A more holistic perspective on interspecific competition: integrating the old with the new

The emergence of non-traditional mechanisms promoting competition in modern ecology is apparent, not only in phytophagous insects, but in a wide range of taxa and ecosystems. The mechanisms underlying competition in plant assemblages, for example, are being challenged by facilitation (Stachowicz 2001, Bruno et al. 2003), and indirect interactions mediated by herbivores (i.e., associational resistance and susceptibility; Rand 2003). Analogous processes have also recently been uncovered and emphasized in marine (Long et al. 2007) and mammalian systems (Roemer et al. 2002). Thus, given the ubiquitous presence of non-traditional forms of competition in phytophagous insects, as well as other animal and plant groups, defining a new paradigm for interspecific competition should be a priority for the fields of population and community ecology (e.g., Bruno et al. 2003).

Based on the above analyses, current competition theory inadequately predicts the nature of interactions occurring in herbivorous insects; virtually every fundamental paradigm that I tested was violated to some degree, suggesting a poor concordance

between theory and empirical patterns. Although advancement in science typically occurs when traditional theories are restructured or otherwise modified to account for newer findings, the extreme disparity between theory and empirical results for phytophagous insects leads me to question if and when the historical perspective on competition applies. More specifically, the conceptual basis for traditional, niche-based competition theory is simply too restrictive to adequately account for current findings, at least those involving insect herbivores. Lawton and Strong (1981) expressed a similar concern years ago with regard to traditional competition and niche theory and their ability to predict interactions among insect herbivores.

Indirect herbivore interactions via plants and enemies are likely to underlie much of the discrepancy between theory and pattern. Until recently most ecology texts emphasized interference and exploitative interactions as the two mechanisms driving competition. My dataset provides weak support for the overall prevalence of these two mechanisms occurring in phytophagous insect communities (Figure 1.9). Alternatively, indirect interactions provide the vast majority of evidence for interspecific herbivore interactions (>65% of all observations in the dataset), particularly those involving plants. The frequency of enemy-mediated interactions, however, is likely to be highly underestimated in because the response variables used in many studies on apparent competition (e.g., % parasitism) could not be integrated into the analyses. Similarly, facilitation, although accounting for >15% of all interactions, may be underestimated because many cases of positive interspecific effects quantify diversity rather than herbivore preference or performance (e.g., Lill and Marquis 2003, Marquis and Lill 2007). Thus, despite the fact that indirect interactions and facilitation are already well-

represented in the dataset, their frequencies provide a conservative estimate of their prevalence in insect communities.

An additional component of indirect plant- and enemy-mediated interactions is that they often impact the entire community of herbivores, leading to more diffuse interspecific effects (Thaler et al. 2001, Van Zandt and Agrawal 2004b, Agrawal 2005, Kaplan and Eubanks 2005, Viswanathan et al. 2005), in contrast with the traditional focus on pair-wise species interactions. Moreover, natural enemies can exacerbate plant-mediated effects between herbivores, a community-level interaction that remains unexplored in most systems. For example, previous feeding by potato leafhoppers (*Empoasca fabae*) results in adverse plant-mediated effects on other herbivores (e.g., slow growth), which in turn dramatically increases their risk of predation (Kaplan et al. 2007). As a result, the new paradigm for interspecific competition must take a community-wide perspective that recognizes positive as well as negative interactions that propagate from both the bottom and top of trophic webs. Furthermore, these interspecific effects are predicted to be highly asymmetric, occur at relatively low herbivore densities, and among divergent taxa that feed at different times and on different plant tissues. A major consequence of this approach is that niche overlap, at least as historically defined, will play a decreasingly important role. Although this paradigm is not likely to be what Darwin and other early ecologists had envisioned in the development of competition theory, it should be central to any current perspective on interspecific interactions involving herbivorous insects, and perhaps other trophic and taxonomic groups.

Figure Legends

Figure 1.1. The distribution of effect sizes (Hedges' d) across studies that quantify the impact of interspecific competition on herbivore (A) development time, (B) relative growth rate, (C) body size, (D) survival, (E) oviposition preference, (F) fecundity, (G) abundance, and (H) plant damage. The data are arranged in rank order such that each point represents the effect size and variance for one observation in the dataset. d_{++} = grand mean effect size, CI = 95% bootstrap confidence intervals, n = number of observations.

Figure 1.2. The impact of defoliation intensity by herbivore species 1 on the preference, performance, and/or damage inflicted by herbivore species 2. *Theory predicts that interspecific competition is directly proportional to the quantity of resources removed.* Therefore, the expectation would be that higher levels of defoliation lead to greater adverse effects on competing species (i.e., a negative relationship between defoliation and effect size).

Figure 1.3. The effects of intra- and interspecific competition on herbivore (A) growth rate, (B) body size, (C) survival, (D) oviposition preference, (E) fecundity, (F) emigration, and (G) composite response. *Theory predicts that intraspecific competition will be greater in magnitude than interspecific competition.* Because of small sample size, development time and relative growth rate were merged to form the single response variable 'growth rate'. Mean effect sizes are presented with 95% bootstrap confidence intervals, and numbers below error bars

are the number of observations per group. None of the comparisons was significant.

Figure 1.4. Consequences of phylogenetic relatedness among interspecific competitors on the strength of competitive interactions. Separate analyses were conducted for interactions occurring within (A) chewing, and (B) sap-feeding guilds. Genus, family, order, and class represent the lowest possible shared taxonomic classification among competitors. For example, the group ‘genus’ consists of competing congeneric pairs (e.g., *Prokelisia dolus* and *Prokelisia marginata*). Similarly, ‘family’ signifies competitors in the same family, but not genus (e.g., *Myzus persicae* and *Aphis gossypii*; both in the family Aphididae, but different genera). I could not compare cross-order interactions (‘class’ grouping) for sap-feeders because all published accounts were within the order Hemiptera. ***Theory predicts that closely-related species will compete more intensely than distantly-related species.*** As a result, effect sizes should be greatest among congeners and weakest in the grouping ‘class’ (cross-order interactions). Mean effect sizes are presented with 95% bootstrap confidence intervals, and numbers below error bars are the number of observations per group. * = significant difference ($P < 0.01$) between groups.

Figure 1.5. The effects of within and across feeding guild interactions on herbivore (A) development time, (B) relative growth rate, (C) body size, (D) survival, (E) oviposition preference, (F) abundance, (G) plant damage, and (H) composite

response. Intra-guild interactions are those occurring between herbivores in the same feeding guild (e.g., chewer on chewer, or sap-feeder on sap-feeder). Inter-guild interactions occur among herbivores in different feeding guilds (e.g., chewer on sap-feeder, or sap-feeder on chewer). ***Theory predicts that within guild competition will be stronger than competition between guilds.*** Mean effect sizes are presented with 95% bootstrap confidence intervals, and numbers below error bars are the number of observations per group. * = significant difference ($P < 0.01$) between groups.

Figure 1.6. The impact of spatial and/or temporal separation on the strength of interspecific competition, including effects on herbivore (A) growth rate, (B) body size, (C) survival, (D) fecundity, (E) abundance, and (F) composite response. Interactions were classified as occurring among herbivores that are (1) not separated from one another, (2) spatially separated (e.g., leaf- and root-feeders) but occur on the plant at the same time, (3) temporally separated (e.g., early- and late-season species) but share the same plant part, and (4) spatially and temporally separated. ***Theory predicts that spatiotemporal co-occurrence will promote more intense competition.*** Because of small sample size, development time and relative growth rate were merged to form the single response variable ‘growth rate’. Mean effect sizes are presented with 95% bootstrap confidence intervals, and numbers below error bars are the number of observations per group. * = significant difference ($P < 0.01$) between groups.

Figure 1.7. The symmetry of reciprocal pair-wise interactions between competing herbivores. The effects of dominant and inferior competitors were reported on herbivore (A) growth rate, (B) body size, (C) survival, (D) fecundity, and (E) abundance. Reciprocal interactions (i.e., effects of sp. 1 on 2, and sp. 2 on 1) were paired and the larger of the two effect sizes was used to define the dominant competitor. The smaller effect size defined the inferior competitor. *If interactions are symmetrical, as predicted by competition theory, then these two groups should be equal in magnitude.* Because of small sample size, development time and relative growth rate were merged to form the single response variable ‘growth rate’. Mean effect sizes are presented with 95% bootstrap confidence intervals, and numbers below error bars are the number of observations per group. * = significant difference ($P < 0.01$) between groups.

Figure 1.8. The symmetry of reciprocal pair-wise interactions between competing herbivores. The effects of dominant and inferior competitors were reported on herbivore composite response for (A) all interactions, (B) direct interactions, and (C) indirect interactions. Reciprocal interactions (i.e., effects of sp. 1 on 2, and sp. 2 on 1) were paired and the larger of the two effect sizes was used to define the dominant competitor. The smaller effect size defined the inferior competitor. *If interactions are symmetrical, as predicted by competition theory, then these two groups should be equal in magnitude.* Mean effect sizes are presented with 95% bootstrap confidence intervals, and numbers below error bars are the number of observations per group. * = significant difference ($P < 0.01$) between groups.

Figure 1.9. Potential mechanisms underlying competitive and facilitative interactions between herbivores, including (A) interference competition, (B) exploitative competition, (C) plant-mediated interactions, and (D) enemy-mediated interactions. Frequencies represent the proportion of all interactions in the dataset that correspond to each category (i.e., interference competition = 12.4% = 30 observations of interference / 243 total observations of significant interspecific interactions). Direct effects are indicated by solid lines, whereas indirect effects are signified by dashed lines. NE = natural enemies, H = herbivores, P = plants, - = competition, + = facilitation.

Figure 1.1

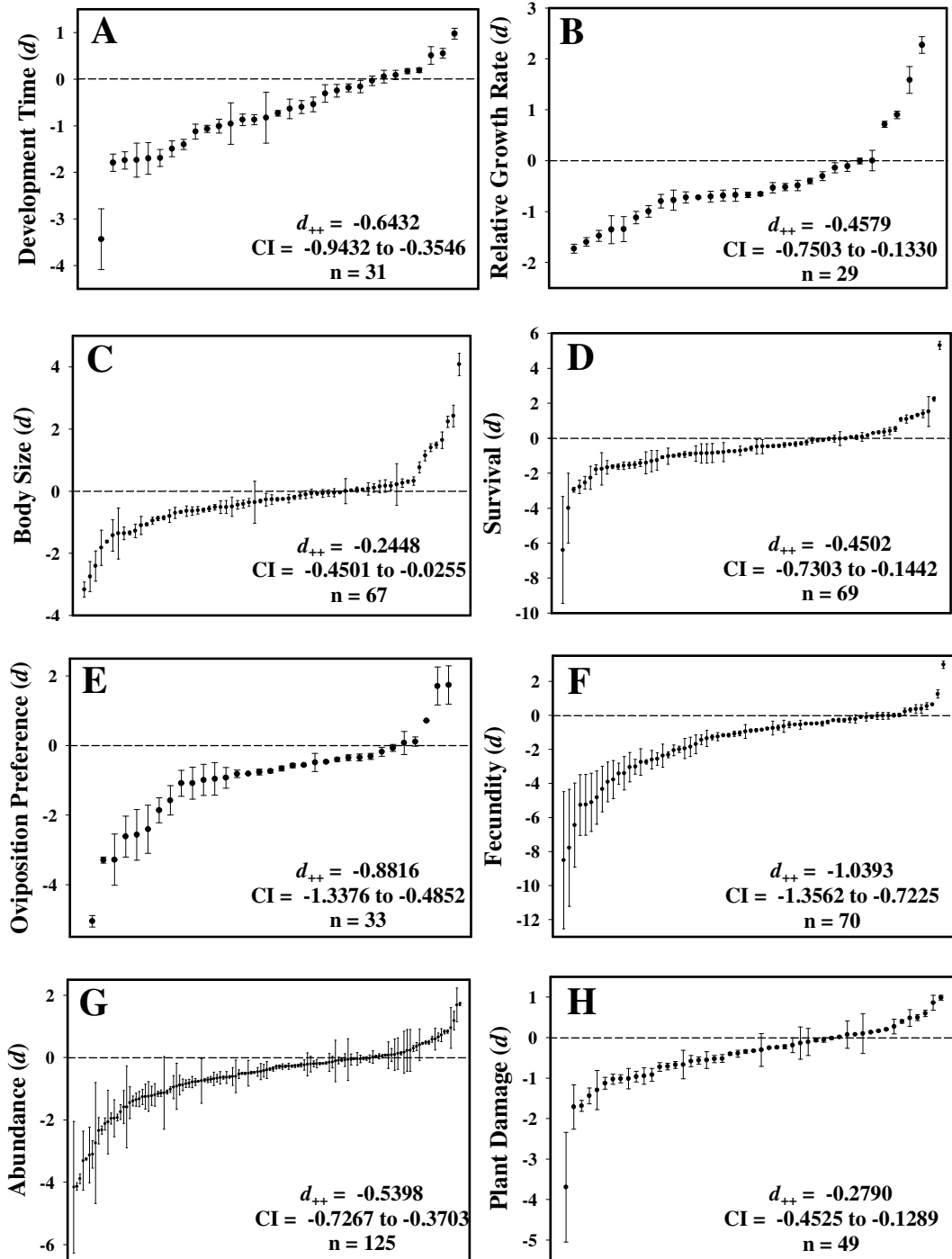


Figure 1.2

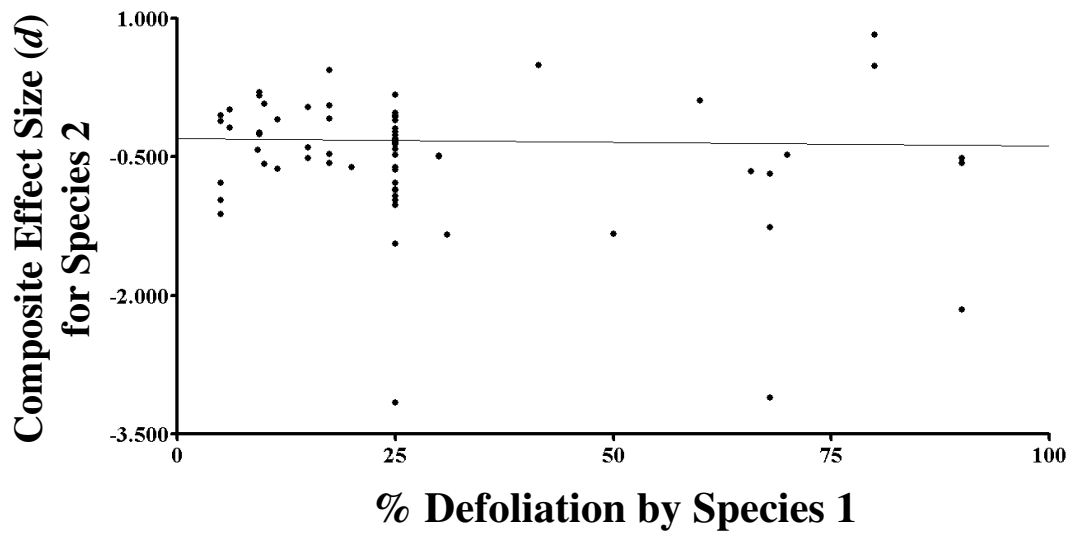


Figure 1.3

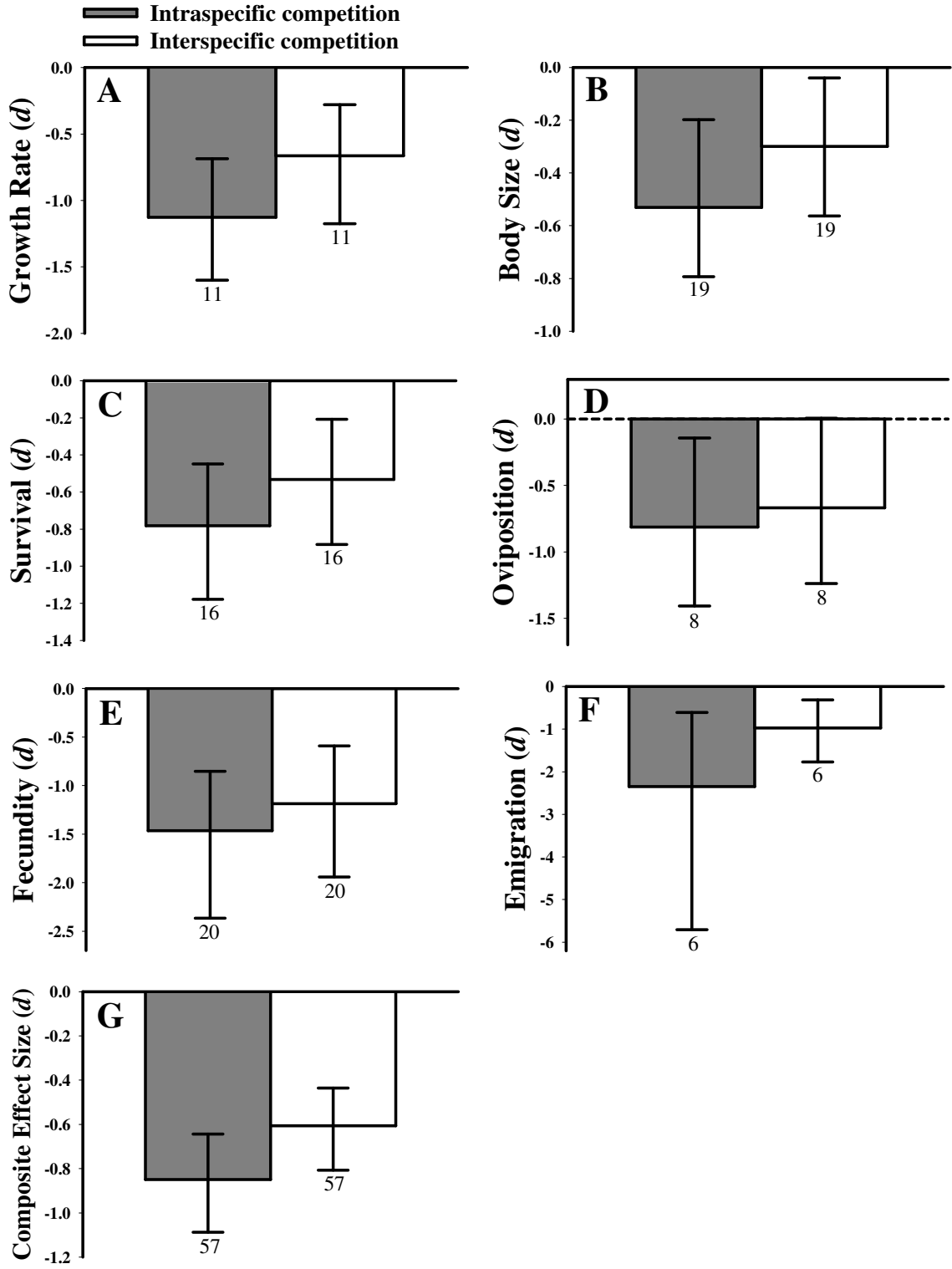


Figure 1.4

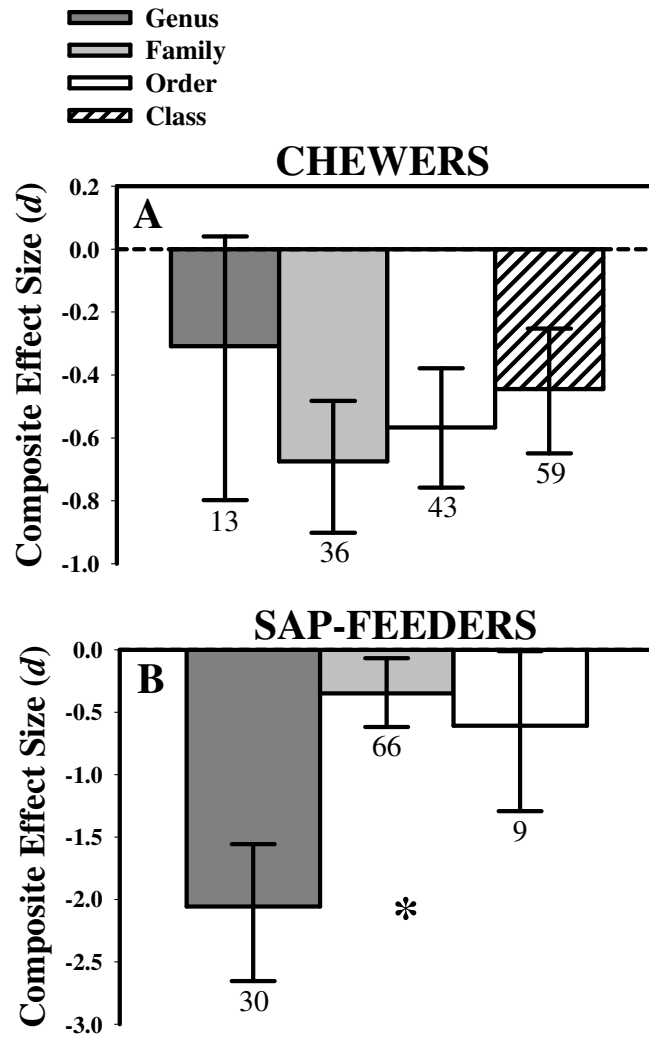


Figure 1.5

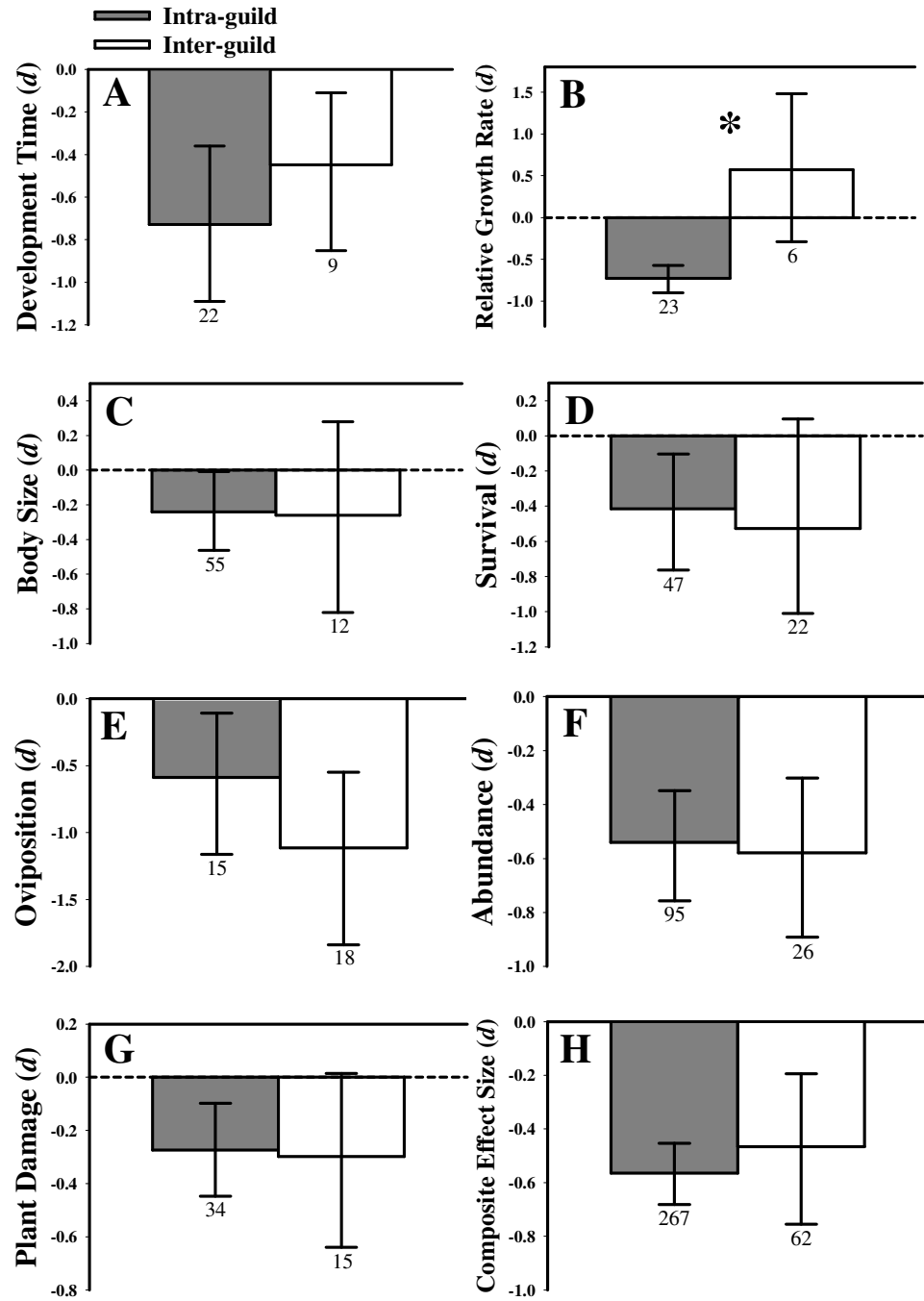


Figure 1.6

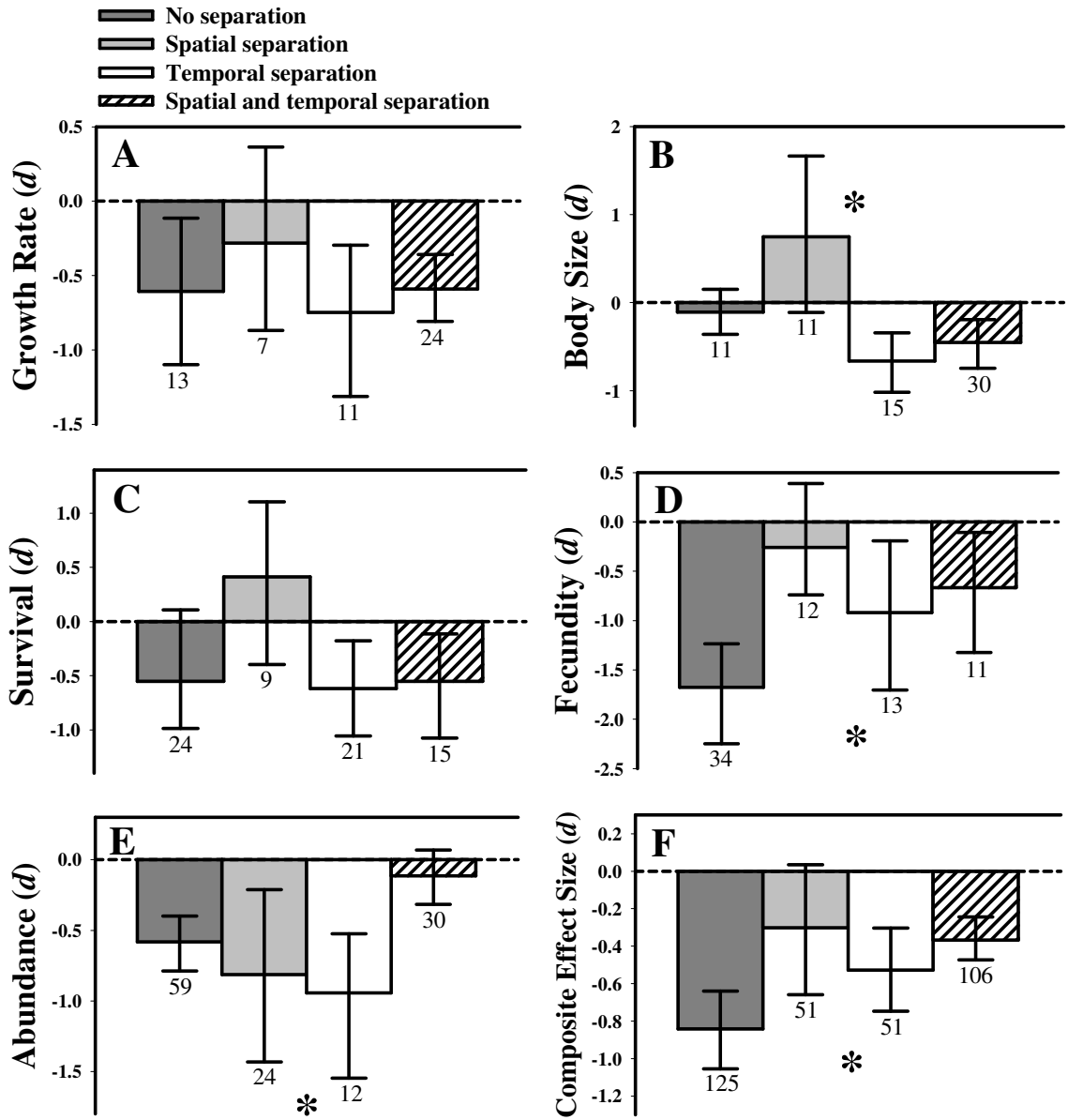


Figure 1.7

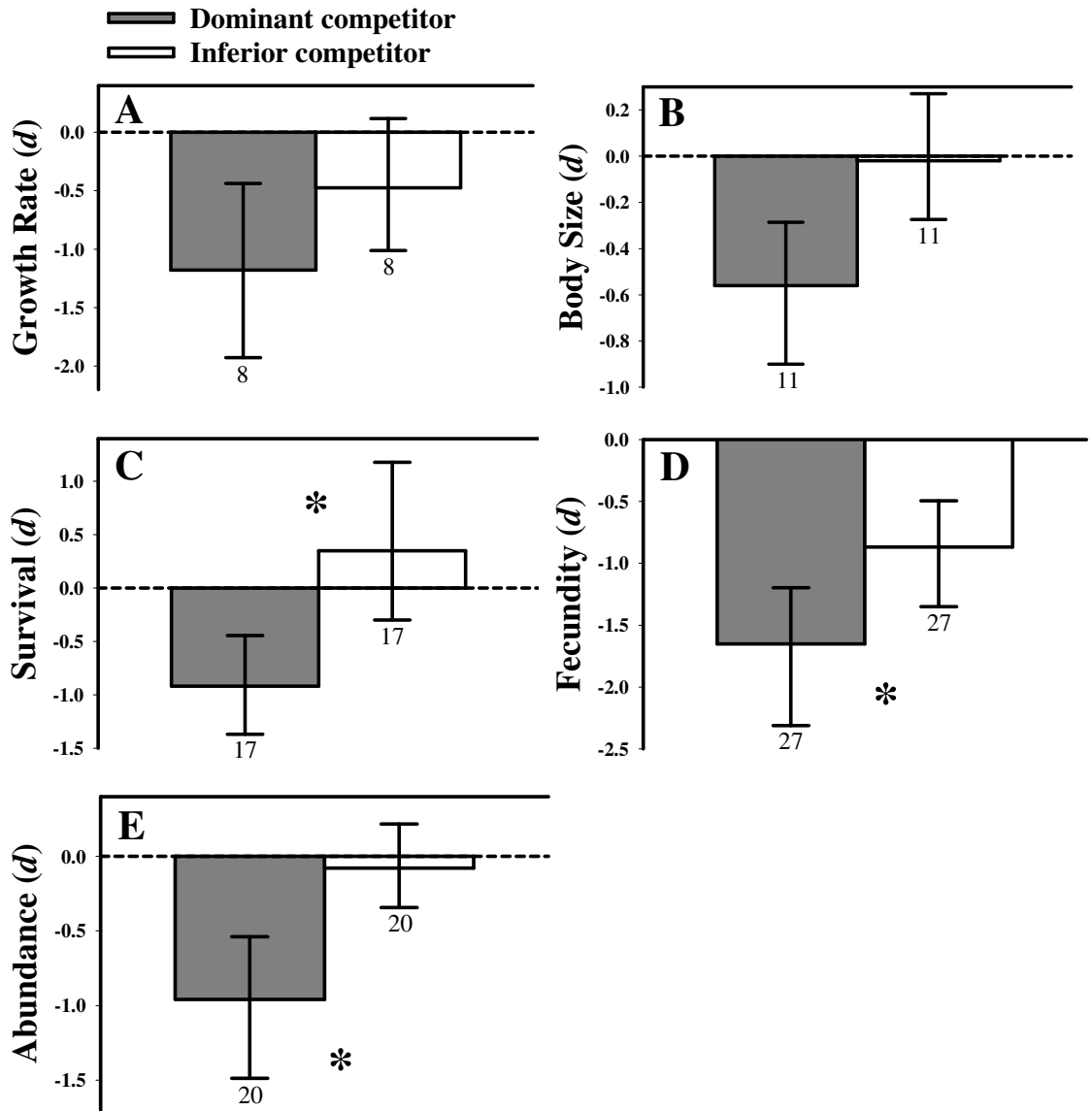


Figure 1.8

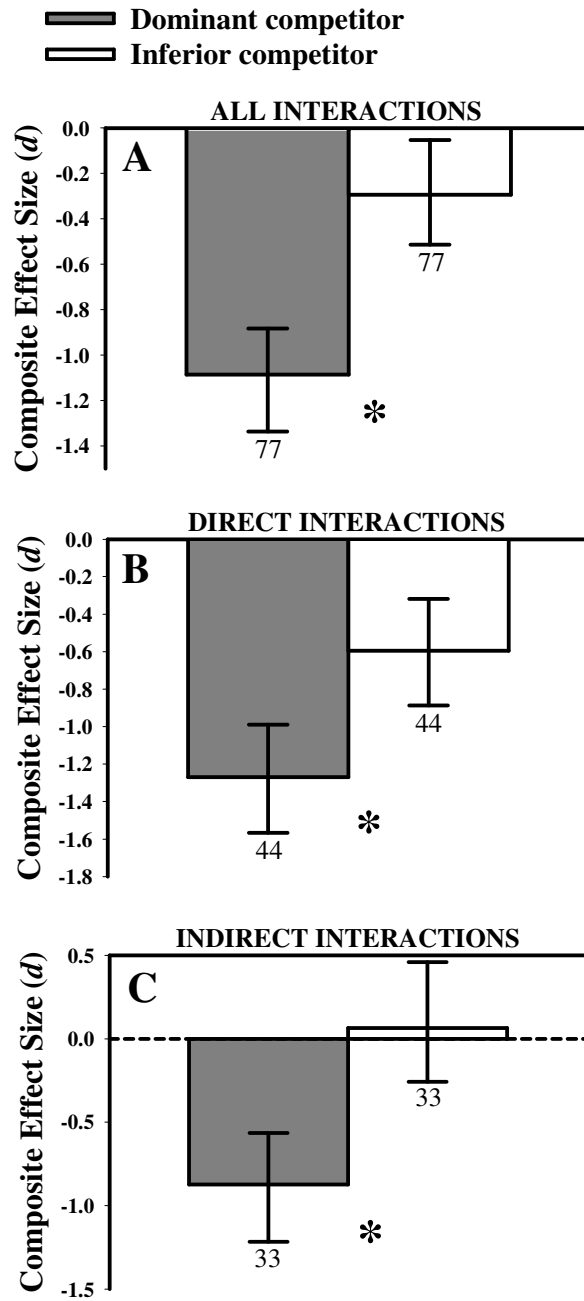
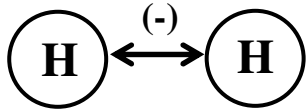
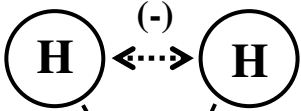
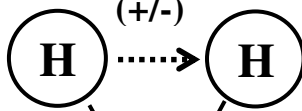
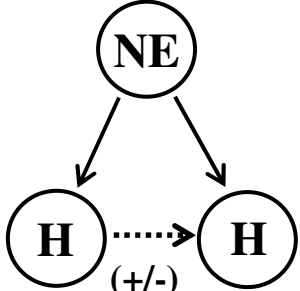


Figure 1.9

	(A) <i>Interference competition</i>	(B) <i>Exploitative competition</i>	(C) <i>Plant-mediated interaction</i>	(D) <i>Enemy-mediated interaction</i>
<i>Trophic level</i>				
<i>3rd</i>				NE
<i>2nd</i>				
<i>1st</i>		P	P	
Mechanism:	Territorial defense; direct aggression; fighting for resources	Indirect interaction involving changes in the <i>quantity</i> of plant tissue	Indirect interaction involving changes in the <i>quality</i> of plant tissue	Indirect interaction via predation or parasitism by natural enemies
Frequency of Competition:	30/243 (12.4 %)	47/243 (19.3 %)	116/243 (47.7 %)	12/243 (4.9 %)
Frequency of Facilitation:	–	–	32/243 (13.2 %)	6/243 (2.5 %)

Chapter 2: Constitutive and induced defenses to herbivory in above- and belowground plant tissues

Abstract

A recent surge in attention devoted to the ecology of soil biota has prompted interest in quantifying similarities and differences between interactions occurring in above- and belowground communities. Furthermore, linkages that interconnect the dynamics of these two spatially distinct ecosystems are increasingly documented. I use a similar approach in the context of understanding plant defenses to herbivory, including how they are allocated between leaves and roots (constitutive defenses), and potential cross-system linkages (induced defenses). To explore these issues I utilized three different empirical approaches. First, I manipulated foliar and root herbivory on tobacco (*Nicotiana tabacum*) and measured changes in the secondary chemistry of above- and belowground tissues. Second, I reviewed published studies that compared levels of secondary chemistry between leaves and roots to determine how plants distribute their defenses across the above- and belowground systems. Last, I used meta-analysis to quantify the impact of induced defenses across plant tissue types.

In the tobacco system, leaf-chewing insects strongly induced higher levels of secondary metabolites in leaves, but had no impact on root defense compounds. Nematode root herbivores, however, elicited changes in both leaves and roots. Virtually all secondary chemicals measured were elevated in nematode-induced galls, whereas the impact of root herbivory on foliar defense was highly variable and depended on where allelochemicals were produced within the plant. Importantly, nematodes interfered with

aboveground defenses that have biosynthetic sites located in roots (e.g., nicotine), but had the opposite effect (i.e., nematodes elevated foliar expression) on chemicals produced in shoots (e.g., phenolics and terpenoids).

Results from the literature review suggest that, overall, constitutive defense levels are extremely similar when comparing leaves with roots, although certain chemical classes (e.g., alkaloids, glucosinolates) are differentially allocated between above- and belowground parts. Based on a meta-analysis of induced defense studies I conclude that: (1) foliar induction generates strong responses in leaves, but much weaker responses in roots, and (2) root induction elicits elevated defenses of equal magnitude in both leaves and roots. I discuss the importance of this asymmetry and the paradox of cross-system induction in relation to optimal defense theory and interactions between above- and belowground herbivory.

Introduction

Optimal defense theory (ODT) was originally developed to explain the ecological and evolutionary forces that generate within-plant heterogeneity in the distribution of defenses against herbivory (McKey 1974, 1979, Rhoades 1979, Denno and McClure 1983). The basic premises of ODT are that defenses are costly to plants and thus should be preferentially allocated to parts that are of greatest value and risk of attack from consumers (Zangerl and Bazzaz 1992). Consequently, ODT can be used to predict the relative expression of defenses in different parts of the same plant. For example, young leaves are considered more valuable than old leaves and often possess far greater concentrations of secondary metabolites (Krischik and Denno 1983, Raupp and Denno

1983, van Dam et al. 1996, de Boer 1999, Ohnmeiss and Baldwin 2000). Similarly, flowers and fruits are more difficult to replace than vegetative parts and frequently possess high levels of resistance to herbivores (Euler and Baldwin 1996, Zangerl and Rutledge 1996, Darrow and Bowers 1999, Kozukue et al. 2004).

An explicit consideration of how plant defenses are differentially allocated between leaves and roots, in the context of ODT, is more difficult to reconcile. Leaves and roots both serve essential functions, with leaves fixing carbon and roots providing water and nutrients. Therefore, leaves and roots, overall, must be considered of approximately equal value to the plant (Zangerl and Bazzaz 1992). However, it should be noted that the value, and thus defense, of above- and belowground parts will likely vary with growing environment. For instance, leaves may be of greater value to plants exposed to low light conditions, whereas roots might be more valuable when water or nutrients are the factors most limiting to plant growth. Additionally, leaves and roots are likely under comparable risk of attack from consumers (Zangerl and Bazzaz 1992; but see Zangerl and Rutledge 1996). Although aboveground herbivory is more widely documented, belowground herbivory is probably underestimated because of the difficulties associated with quantifying root damage (Brown and Gange 1990, Hunter 2001). Furthermore, a diverse assemblage of root-feeding organisms (e.g., nematodes, fungi) is well-adapted for the soil environment (Coleman et al. 2004). Thus, because leaves and roots are equally valuable to plants and experience similar risk of attack from consumers, ODT predicts that constitutive defenses, on average, should be similarly apportioned above- and belowground.

Inducible plant defenses, however, provide a more intriguing scenario. In order to predict where plant defenses will be expressed in response to above- and belowground herbivory, ODT relies primarily on the risk of future attack (i.e., there is no *a priori* reason to assume that the relative value of leaves vs. roots will change greatly in the presence of herbivores). When an herbivore initiates feeding on a plant leaf, for example, defenses are often induced locally in the damaged leaf as well as systemically in undamaged leaves (Karban and Baldwin 1997). From the plant's perspective this allocation pattern may be adaptive given that small amounts of leaf damage likely forecast additional leaf removal to herbivores in the near future. Consequently, defoliation places leaves under heightened risk of subsequent attack. The majority of foliar-feeding herbivores, however, do not simultaneously consume belowground tissues and therefore, in most instances, defoliation should not greatly alter the risk of attack to plant roots (Brown and Gange 1990, Coleman et al. 2004). Accordingly, foliar herbivory should elicit increased defenses in leaves and little-to-no changes in the defensive phenotype of roots, and vice versa. In other words, ODT predicts elevated resistance in the sub-system under attack (e.g., roots in response to belowground herbivory) with no corresponding change in spatially separated plant parts that are not at risk of attack (e.g., leaves in response to belowground herbivory).

Despite the above predictions regarding defense induction between leaves and roots, we know from recent studies that cross-system induction is indeed possible (see reviews by Bezemer and van Dam 2005, van Dam and Bezemer 2006). For instance, beetle larvae feeding on roots of *Gossypium herbaceum* elicit a >4-fold increase in the concentration of terpenoids in mature leaves (Bezemer et al. 2003, 2004). Superficially,

this appears to violate one of the fundamental tenets of optimal defense. If plants are ‘smart investors’ (*sensu* van Dam et al. 1996), then why would costly defenses be expressed far from the site of attack where they seemingly serve no purpose?

Furthermore, many plants produce aboveground secondary chemicals in their roots, and vice versa (Karban and Baldwin 1997, van der Putten et al. 2001, Bezemer et al. 2003, van Dam et al. 2003). For example, tobacco alkaloids are considered highly effective defenses against foliar herbivores, yet these compounds are synthesized entirely belowground (Dawson 1941, 1942, Baldwin 1991). Therefore, the magnitude and direction of induced responses to foliar and root herbivores may depend strongly on where plants produce secondary metabolites and the degree to which herbivores damage such tissues (i.e., root herbivores on tobacco may interfere with foliar alkaloid accumulation).

To consider the paradox of cross-system induction and other issues (i.e., the importance of variation in biosynthetic sites for secondary metabolite induction) related to constitutive and induced defenses in an above/belowground context I provide two different empirical approaches. First, I experimentally test the impact of foliar and root herbivory on whole-plant secondary metabolite expression using tobacco (*Nicotiana tabacum*) as a model system. More specifically, I sought to determine if and how root-derived phytochemicals (e.g., alkaloids) differ from foliar-derived chemicals (e.g., phenolics) in their response to herbivore damage. Second, I conduct a review of the available literature comparing levels of constitutive and induced defenses in leaves and roots. Although several outstanding reviews have recently been published on herbivore-induced responses linking above- and belowground plant parts (van der Putten et al.

2001, Blossey and Hunt-Joshi 2003, van Dam et al. 2003, Bezemer and van Dam 2005, van Dam and Bezemer 2006), this is the first quantitative assessment of the topic.

Specifically, I was interested in answering the following questions: (1) do constitutive defenses differ between leaves and roots, and (2) how does the magnitude of induction compare between herbivory in the tissue being attacked (i.e., leaves in response to foliar herbivores) versus the opposing tissue type (i.e., roots in response to foliar herbivores)?

Methods

Study system

Tobacco (*N. tabacum*) is a perennial plant native to South America that is widely cultivated in the mid-Atlantic United States. In this study, I used an agricultural variety of tobacco, and consequently the expression of secondary metabolites may be influenced by the fact that tobacco has long been selected for various agronomic traits (e.g., foliar nicotine concentration).

Plants in the genus *Nicotiana* have been among the most well-studied with regard to constitutive and induced defenses to herbivory (Karban and Baldwin 1997, Steppuhn et al. 2004). Several major classes of tobacco secondary chemicals are inducible and have been implicated in reducing the preference and/or performance of herbivores, including: alkaloids (e.g., nicotine; Baldwin 1988a, Krischik et al. 1991, Jackson et al. 2002, Steppuhn et al. 2004), phenolics (e.g., chlorogenic acid, rutin; Krischik et al. 1991, Stamp et al. 1994, Pegard et al. 2005), and terpenoids (e.g., diterpene glycosides; Snook et al. 1997, Jassbi et al. 2006). The alkaloid nicotine, in particular, is a highly inducible compound that has been one of the most heavily studied chemicals in relation to

herbivore resistance (Schmeltz 1971, Baldwin 1991, Steppuhn et al. 2004). Notably, nicotine biosynthetic sites are located entirely belowground in growing root tips and nicotine is subsequently translocated aboveground where it protects leaves from insect defoliation (Dawson 1941, 1942, Baldwin 1991). As a result, nicotine provides a potentially important link between the above- and belowground systems.

The foliar-feeding herbivores of tobacco include a diverse assemblage of leaf-chewing insects such as *Manduca sexta* and *Trichoplusia ni*. The tobacco hornworm, *M. sexta*, is an oligophagous caterpillar that specializes on solanaceous plants (particularly *Nicotiana* sp.) and therefore has physiological adaptations for tolerating alkaloids (Wink and Theile 2002). Moreover, *M. sexta* is considered a “stealthy” herbivore because it attenuates the induced nicotine response that occurs following leaf damage (McCloud and Baldwin 1997, Kahl et al. 2000, Winz and Baldwin 2001). Consequently, these caterpillars often have dramatic effects on their host and can defoliate entire plants. The cabbage looper, *T. ni*, is a polyphagous caterpillar that feeds from >25 different plant families (Soo Hoo et al. 1984), and tobacco secondary chemicals are known to strongly reduce the performance of this insect (Krischik et al. 1991).

The root-knot nematode, *Meloidogyne incognita*, is considered the dominant belowground plant parasite in tobacco-growing regions (Barker and Lucas 1984). This species is among the most economically important group of plant-parasitic nematodes worldwide, largely because of its extraordinarily broad host range (Trudgill and Blok 2001). *M. incognita*, along with other phytoparasitic nematodes, feed on cellular contents and *Meloidogyne* sp. also induces the development of galls at feeding sites in root tissue.

Herbivore-induced foliar and root chemistry in Nicotiana tabacum

I tested the individual and combined effects of above- and belowground herbivory on secondary metabolite expression in tobacco leaves and roots. Foliar herbivory was manipulated by adding caterpillars to leaves (3 treatments: undamaged control, *M. sexta* defoliation, and *T. ni* defoliation), while root herbivory was manipulated by inoculating roots with nematodes (2 treatments: undamaged control, and *M. incognita* inoculation). These treatments were randomly assigned to individual plants (= unit of replication) in a fully-crossed block design (3 foliar x 2 root = 6 treatments; n = 10-15 replications per treatment combination).

Tobacco plants (var. MD 609) were propagated by seed in a greenhouse and seedlings (9 weeks of growth from seed to seedling stage) were subsequently transplanted into 4-gallon pots containing a sterilized growing medium [50% sand, 50% potting mix (SunGro LC1 and professional blend; sphagnum peat moss, bark, perlite, vermiculite, and clay)]. Plants were supplemented with nutrients weekly using a soluble fertilizer (20:10:20 NPK). The roots of all seedlings assigned to root herbivory were inoculated at the time of transplant with 100,000 *M. incognita* eggs obtained from a laboratory culture (Sardanelli and Kenworthy 1997). This egg density is well within the range documented for *M. incognita*-infested tobacco fields (Barker and Lucas 1984) and also corresponds with inoculum levels used in prior studies (Hanounik and Osbourne 1975, 1977, Barker and Weeks 1991, Wheeler et al. 1991, Vovlas et al. 2004). Foliar herbivory was inflicted on nematode-infested and nematode-free plants 4 weeks after inoculation. This time period allowed *M. incognita* eggs to hatch, juveniles to penetrate roots and induce gall formation, yet plants remained in the rosette stage and thus were still inducible

(Ohnmeiss and Baldwin 2000, van Dam et al. 2001). Caterpillars were placed in mesh sleeve-cages (one 2nd instar *M. sexta* or six 3rd instar *T. ni*) on the 2 youngest fully expanded leaves and remained until both leaves were entirely defoliated (8 days).

Four days after caterpillars were removed, coinciding with peak induction of foliar nicotine (Baldwin 1991), plants were harvested for chemical analyses. Leaf [1st newly expanded (i.e., youngest) leaf] and root tissue samples were collected from all plants, while *M. incognita*-induced galls were harvested separately from non-galled root tissue on nematode-inoculated plants. Samples were immediately frozen in liquid nitrogen and extracted and analyzed by HPLC (Keinänen et al. 2001) on a reversed phase C18 column (Gemini C18, 150 x 4.6 mm; Phenomenex). Concentrations of identified alkaloids and phenolics were quantified using calibration curves prepared from commercially available standards. Concentrations of diterpene glycosides (DTGs) and two unknown caffeic acid-derived phenolics were calculated from peak areas at 210 nm (for DTGs) and 320 nm (for phenolics).

The impact of foliar and root herbivory on above- and belowground plant chemistry was first tested using a three-way MANOVA on leaf, root, and gall tissues with nematodes, *M. sexta*, and *T. ni* as main effects (proc glm; statistical analyses were performed using SAS, Version 9.1; SAS Institute, Inc., 2001). Interactive effects were examined between nematodes and each individual caterpillar species (the full model was not tested because the design did not include a treatment with damage by both caterpillar species on the same plant). Next I used univariate ANOVAs (same as above, 3-way with all 3 herbivores as fixed effects) for each chemical measured in each tissue type; block (i.e., spatial groupings of plants on benches) was considered as a random effect in the

model (proc mixed). Data were transformed (square root and log transformations) as needed to meet assumptions of normality and homogeneity of variances.

Two additional pre-planned comparisons not included in the above analyses were made to separate potential differences between the 2 caterpillar species (i.e., Does the generalist have a different impact on plant chemistry than the specialist?) and galled vs. non-galled tissues (i.e., Does the concentration of secondary chemicals in galls differ from adjacent non-galled root tissue?). Similar to the above analyses I used MANOVA followed by univariate ANOVAs to assess the impact of each factor. To avoid interactive effects with nematodes, I only used data from nematode-free plants to quantify the difference between generalist and specialist caterpillars (using the full dataset does not qualitatively change the outcome).

Because the chemicals measured in this study have biosynthetic sites that are spatially separated from one another, I predicted that this variation would explain the direction and magnitude of aboveground plant responses to root herbivores. Tobacco alkaloids are synthesized in the roots (Baldwin 1988b, 1989, Baldwin et al. 1994, Zhang and Baldwin 1997, Nugroho and Verpoorte 2002), whereas DTGs and phenolics are produced in the shoots (Keene and Wagner 1985, Shirley 1996, Harborne and Williams 2000, Tattini et al. 2000, Nugroho and Verpoorte 2002, Roda et al. 2003). Therefore, I used meta-analysis to quantify the impact of root vs. shoot production of defense chemistry on nematode-induced changes in aboveground defenses in tobacco (see below for details on meta-analysis procedures).

Constitutive and inducible plant defenses in leaves and roots: literature review

The literature on constitutive (i.e., undamaged plants) and inducible defenses in leaves and roots was reviewed to: (1) compare constitutive plant defenses between foliar and root tissues; and (2) quantify the magnitude of induction in the plant sub-system under attack (e.g., roots in response to root herbivores, or leaves in response to foliar herbivores) vs. spatially separated parts (e.g., leaves in response to root herbivores, or roots in response to foliar herbivores). To find literature relevant to these two issues I first surveyed the cited works from recently published reviews on leaf and root defenses (e.g., constitutive: Table 16.2, Zangerl and Bazzaz 1992; induced: Table 1, van Dam and Bezemer 2006). Additionally, I performed keyword searches in the database Web of Science using various combinations of the following terms: herbivore, insect, nematode, pathogen, chemistry, defense, constitutive, induced, secondary plant metabolite, foliar, leaves, roots, aboveground and belowground.

The dataset was restricted to studies that measured secondary plant metabolites in leaves and roots. Other plant resistance traits that are only expressed above- or belowground (e.g., trichomes, extrafloral nectar) were not considered. Because the purpose of this analysis was to examine differential allocation patterns I restricted the search to compounds that were shared between leaves and roots. In other words, if a chemical was detected in leaves, but not in roots (or vice versa) then it was excluded from the dataset. This corrects for differences in the capacity to express compounds that are unrelated to allocation. For example, certain chemicals are only found in glandular trichomes on the leaf surface and thus can not be expressed in roots (Roda et al. 2003).

For constitutive defense papers I gathered publications that quantified the concentration of secondary plant metabolites in both leaf and root tissues. I then used

these data to calculate the proportion of secondary chemicals that are expressed above- and belowground [i.e., proportion in leaves = concentration in leaves / (concentration in leaves + concentration in roots)]. In this case the proportion represents a quantitative estimate of the relative measure of defense when comparing leaves with roots; higher concentrations of secondary chemicals in leaves results in a lower proportion in the roots, and vice versa. As a result, we assume that one unit of defense compound is equally effective in roots and shoots.

Each unique chemical-plant species combination was considered an observation. In other words, if a study reported data on 5 different secondary chemicals in the leaves and roots from a single species of plant, these were considered separate observations in the dataset. This unit of replication is justified given that above- and belowground allocation can vary drastically among different chemicals, even within the same plant and/or class of chemistry. We calculated average foliar and root concentrations from studies that varied plant genotype/variety or manipulated environmental factors (e.g., light, nutrients). The proportion of secondary chemicals in foliar vs. root tissues was tested against the null hypothesis of equal distribution (i.e., proportion in leaves and roots = 0.5) using a t-test. This was assessed for the full dataset (total secondary metabolites) and individually for classes of chemicals that were well-replicated (>5 studies). Arcsine square-root transformations were performed on proportional data prior to statistical analyses to satisfy normality.

Induced defenses within and across tissue types were investigated using meta-analysis. Therefore, the dataset was restricted to studies that included means, variation (e.g., standard error, standard deviation), and sample size for the control and treatment

groups. Ideally, I sought out studies that induced defenses in both leaves and roots and subsequently measured plant allelochemical responses in leaf and root tissues. However, this type of design has rarely been used. Most studies either manipulated induction in one tissue type (e.g., leaves) and measured the response in leaves and roots, or induced both tissue types (e.g., leaves and roots) and quantified the response in one of the tissues. I used defense induction in its broadest sense to include any organism that attacks plants, including herbivorous insects, pathogens, and nematodes. Additionally, I included studies that applied plant hormones (jasmonic or salicylic acid) known to elicit secondary metabolite responses similar to that observed following actual herbivory (e.g., Thaler et al. 1996). I did not include studies that used artificial (mechanical) damage because of the substantial differences in phytochemical responses that can occur when comparing artificial and actual herbivore damage (Karban and Baldwin 1997).

Three parameters defined what was considered a replicate in the dataset: (1) the chemical, (2) the inducing agent, and (3) the tissue type. First, as with the constitutive defense dataset, I considered each plant chemical measured as a replicate observation. Second, when studies elicited plant responses using multiple inducing agents (i.e., different species of herbivores) these were also considered separately. Last, if chemicals were measured from distinct tissue classes (e.g., old vs. young leaves, tap vs. fine roots) they remained as separate observations rather than combined. Many studies have now documented that these 3 factors are of paramount importance in explaining variation that underlies plant responses to attack from herbivores (Karban and Baldwin 1997). Therefore, I felt that averaging or otherwise removing this variation from the dataset would ignore the considerable body of information that has accumulated on the dynamics

of induced plant responses. In a similar manner, community ecologists typically split (rather than lump) species into separate observations when using meta-analysis to quantify organismal responses to environmental stimuli (Rosenberg et al. 2000).

Similar to most other ecological meta-analyses I used Hedges' d to calculate effect sizes (Rosenberg et al. 2000). Non-induced plants were designated as the control, induced plants were considered the treatment, and plant secondary chemistry was used as the response variable. Thus, a positive effect size indicates that induction elevated plant allelochemistry and a negative effect size demonstrates a decrease in chemistry. The meta-analytical program *MetaWin 2.0* was used to score effect sizes and conduct the overall analysis (Rosenberg et al. 2000). I used a mixed-effects categorical model to compare effect sizes in the plant tissue being induced vs. the opposing, spatially separated tissue (i.e., If the roots are induced, how do chemical responses compare in the roots vs. the leaves?). For each category a mean effect size (d_+) was calculated and reported with 95% bootstrap confidence intervals; effects are considered significant if confidence intervals do not bracket zero. Between-group heterogeneity (Q_B) was tested against a χ^2 -distribution to determine if significant differences exist among groups of predictor variables.

Results

Herbivore-induced foliar and root chemistry in Nicotiana tabacum

Foliar herbivory by both caterpillar species had no impact on the secondary chemistry of tobacco roots independent of nematode presence (Figure 2.1; *M. sexta*, MANOVA: $F_{6,68} = 1.22$, $P = 0.3061$; *T. ni*, MANOVA: $F_{6,68} = 1.21$, $P = 0.3119$), but

induced higher levels of secondary chemicals in leaves (Figure 2.2; *M. sexta*, MANOVA: $F_{6,71} = 6.73$, $P < 0.0001$; *T. ni*, MANOVA: $F_{6,71} = 32.11$, $P < 0.0001$; see Table 2.1 for univariate ANOVAs). All classes of foliar chemicals were responsive to caterpillar leaf damage, with alkaloids (Figures 2.2A and 2.2B) and DTGs (Figure 2.2F) significantly elevated and inconsistent responses among phenolics (Figures 2.2C-E). Moreover, generalist (*T. ni*) and specialist (*M. sexta*) herbivores had differential effects on foliar chemistry in the absence of nematodes (MANOVA: $F_{6,20} = 8.55$, $P < 0.0001$); in every case the magnitude of induction elicited by the generalist exceeded that of the specialist.

Nematode herbivory affected the secondary chemistry of both non-galled (i.e., systemic) and galled (i.e., local) root tissue independent of caterpillar defoliation [Figure 2.1; MANOVA (non-galled root): $F_{6,68} = 10.74$, $P < 0.0001$; MANOVA (galled root): $F_{6,66} = 220.67$, $P < 0.0001$]. In non-galled tissue the effects of nematodes were variable, ranging from positive (Figures 2.1E and 2.1F) to negative (Figures 2.1A and 2.1B). However, in virtually all instances galls accumulated higher concentrations of secondary chemicals than adjacent roots that were not galled [MANOVA: $F_{6,16} = 22.97$, $P < 0.0001$].

Leaves were similarly affected by root herbivory (Figures 2.2; MANOVA: $F_{6,71} = 20.74$, $P < 0.0001$). Again the outcome of this interaction was variable with nematodes strongly interfering with aboveground alkaloid expression independent of caterpillar damage (Figures 2.2A and 2.2B), but elevating foliar concentrations of phenolics (Figures 2.2C-E) and DTGs (Figure 2.2F). For instance, the addition of nematodes resulted in a >400% increase in foliar chlorogenic acid on caterpillar-free plants. Furthermore, root herbivory modified the response of plants to shoot herbivores. Most

notably, nicotine was increased in response to defoliation by *T. ni* on nematode-free plants, but this inducible response was suppressed on plants that were experimentally inoculated with nematode root herbivores (Figure 2.2A; *T. ni* x *M. incognita* interaction, $F_{1,63} = 7.35$, $P = 0.0087$).

The location of biosynthetic sites for each secondary chemical quantified in this study strongly determined whether root herbivory interfered with or elevated aboveground expression (Figure 2.3). Nematodes induced a decrease in foliar chemistry when considering compounds that are produced in the roots, but elicited higher foliar concentrations of chemicals synthesized in shoots (without aboveground herbivory: $Q_B = 24.22$, $df = 1$, $P < 0.0001$; with aboveground herbivory: $Q_B = 68.26$, $df=1$, $P < 0.0001$).

Constitutive and inducible plant defenses in leaves and roots: literature review

For the database comparing constitutive above- and belowground defenses I accumulated a total of 230 observations obtained from 74 different studies (Appendix A). Overall, there was no difference in the proportion of secondary metabolites allocated between leaves and roots (Figure 2.4; $t = 0.95$, $df = 229$, $P = 0.3452$). However, I detected differential above- and belowground expression for 2 of the 4 classes of chemicals that were investigated in greater detail. Alkaloids occurred at higher concentrations in leaves than roots ($t = 2.98$, $df = 48$, $P = 0.0045$), whereas glucosinolates demonstrated the opposite pattern with higher levels in roots compared with leaves ($t = 3.24$, $df = 68$, $P = 0.0019$). Phenolics ($t = 0.96$, $df = 33$, $P = 0.3430$) and terpenoids ($t = 0.12$, $df = 22$, $P = 0.9082$) were allocated similarly between above- and belowground plant parts.

In the meta-analysis of induced defenses from leaf and root herbivory I calculated effect sizes from 22 studies that provided a total of 315 observations (Appendix A). Regardless of where plants were induced or where the responses were measured, induction elevated the expression of secondary plant chemistry [Figure 2.5; none of the error bars (95% CI) bracket zero]. There were differences, however, in the spatial extent of induction depending on where the response was elicited. Foliar induction resulted in a stronger response in leaves than roots (Figure 2.5A; $Q_B = 5.14$, $P = 0.0234$). Root induction elicited similar effects when comparing the magnitude of expression between leaves and roots (Figure 2.5B; $Q_B = 0.004$, $P = 0.9509$).

Discussion

Above- and belowground induction in Nicotiana tabacum

Caterpillars induced defenses in leaves, but had no measurable impact on tobacco root chemistry (Figures 2.1 and 2.2). Additionally, the generalist *T. ni* tended to elicit stronger responses than the specialist *M. sexta* (nicotine: 42% increase from *M. sexta* vs. 85% increase from *T. ni*; DTGs: 95% increase from *M. sexta* vs. 2400% increase from *T. ni*). Hornworms (*M. sexta*) are known to suppress damage-induced nicotine accumulation in wild tobacco (McCloud and Baldwin 1997, Kahl et al. 2000, Winz and Baldwin 2001), although their impact on DTG expression has not previously been quantified. Nicotine is considered to be a sub-optimal defense against *M. sexta* because the larvae can detoxify and excrete alkaloids (Wink and Theile 2002), perhaps explaining why tobacco plants induce a weak nicotine response to hornworms. Similarly, *M. sexta* is relatively tolerant of diterpenes in their diet (Jassbi et al. 2006), particularly when compared with generalist

caterpillars (Snook et al. 1997). Thus, in a manner analogous to nicotine, tobacco may suppress diterpene accumulation in response to *M. sexta* damage because it is an ineffective resistance mechanism against specialists. Interestingly, DTGs are only mildly increased in leaf tissue after methyl jasmonate application to wild tobacco (1.5- to 1.9-fold increase; Keinänen et al. 2001) suggesting that *T. ni* may greatly amplify the jasmonate-mediated induction. Although specialist herbivores are known to be more tolerant of secondary plant chemistry than generalists, these results add to a growing body of evidence that generalists and specialists elicit very different responses in their host plant (Hartley and Lawton 1987, Bowers and Stamp 1993, Sirvent et al. 2003, Voelckel and Baldwin 2004, Mewis et al. 2006; but see Agrawal 2000, Reymond et al. 2004).

Nematode-induced galls strongly accumulated all classes of chemicals (Figure 2.1). The concentration of rutin, for example, was >8000% higher in galled compared with non-galled roots. These results are in accordance with several studies which have found that herbivore-induced galls possess extraordinarily high quantities of secondary chemicals (Abrahamson et al. 1991, Hartley 1998, Allison and Schultz 2005, Motta et al. 2005). Nematode herbivory, however, did not always result in systemic elevation of root chemistry. In fact, alkaloid concentrations were significantly lower in non-galled roots of nematode-inoculated plants (Figures 2.1A and 2.1B), indicating that allocation of alkaloids to galls may come at the expense of non-galled root tissue.

In marked contrast with caterpillars, nematode root herbivory affected secondary chemistry not only at their feeding sites (i.e., galls) but also aboveground in tobacco leaves (Figure 2.2). I differentiate the impact of root herbivory on foliar chemistry as (1)

direct effects – nematodes alter aboveground chemistry, independent of leaf damage by caterpillars; and (2) *indirect effects* – nematodes influence the magnitude of induction by caterpillars (i.e., statistical interaction between root and shoot herbivory). Both direct and indirect effects of root herbivory were evident, depending on the chemicals measured. Nematodes had negative direct and indirect effects on foliar alkaloids (Figures 2.2A and 2.2B; Hanounik and Osborne 1975, 1977, Barker and Weeks 1991). Aboveground nicotine concentrations, for example, were ~40% lower on plants with root herbivory. Similarly, nicotine was highly inducible by caterpillar defoliation on nematode-free plants yet became almost entirely non-inducible when nematodes were present (*M. sexta*: 42% increase without root herbivory vs. 12% increase with root herbivory; *T. ni*: 85% increase without root herbivory vs. 24% increase with root herbivory).

Unlike alkaloids, root herbivory induced positive direct and indirect effects on tobacco phenolics and terpenoids. Nematodes tended to have positive direct effects on phenolics (i.e., higher foliar concentrations on plants with nematodes alone), but inconsistent and unpredictable indirect effects (Figures 2.2C-E). This result is not entirely surprising given the diverse role that many phenolic compounds play in the biology of plants (Shirley 1996, Harborne and Williams 2000, Roda et al. 2003). In contrast, nematodes had weak direct effects on foliar DTGs, but positive indirect effects (Figure 2.2F). For instance, on nematode-free plants *M. sexta* damage elicited a 95% increase in DTGs, but on plants with nematodes *M. sexta* herbivory resulted in a >500% increase in DTGs. Thus, nematodes alone have weak effects on foliar DTGs, but prime plants for much stronger responses to aboveground caterpillar feeding. This priming phenomenon is known to occur in plant-pathogen interactions (Conrath et al. 2002, 2006) and recent

work suggests that herbivore-induced plant volatiles can prime neighboring plants (Engelberth et al. 2004, Heil and Kost 2006, Kessler et al. 2006, Ton et al. 2006). Additionally, van Dam et al. (2005) demonstrated that the phytoparasitic nematode *Pratylenchus penetrans* primes plants for more rapid phenolic responses to caterpillar herbivory.

Although several studies provide evidence that root herbivores elevate defenses in foliar tissues (see reviews by Bezemer and van Dam 2005, van Dam and Bezemer 2006), my data suggest highly divergent effects of belowground herbivory on aboveground secondary metabolite expression in the tobacco system. Nematodes interfered with certain classes of compounds (e.g., alkaloids), but induced higher levels of defense when assessing other groups of chemicals (e.g., phenolics and DTGs). What factor(s) are responsible for generating this variation? I hypothesize that the location of biosynthetic sites for these diverse phytochemicals is an extremely important consideration. Tobacco alkaloids are known to be synthesized entirely belowground in growing root tips, whereas the phenolics and terpenoids that we measured are produced in leaf tissue. As a result, root herbivory interfered with foliar chemicals that are synthesized in roots and elevated foliar chemicals that are produced aboveground (Figure 2.3).

The importance of this phenomenon was predicted by Karban and Baldwin (1997) who proposed that since “nicotine biosynthesis is located in the roots...a plant’s ability to respond would rapidly diminish with increasing amounts of root herbivory.” The presumed advantage of producing alkaloids in roots is to protect the machinery for launching a sustainable defense reaction in the face of intense defoliation (Baldwin and Schmelz 1994, Karban and Baldwin 1997). However, this also suggests that the alkaloid-

based defense system of tobacco is extremely susceptible to root herbivory. Interestingly, nematodes increased foliar nicotine concentrations on nematode-resistant tobacco varieties (Hanounik and Osborne 1977). Similarly, Preisser et al. (2007) inoculated tobacco roots with low nematode densities and found no impact on leaf nicotine. Thus, the magnitude of damage incurred by roots may ultimately dictate the degree of interference from belowground consumers.

Several recent studies have emphasized the importance of roots as biosynthetic sites for aboveground chemicals (e.g., terpenoids in *Gossypium* sp., furanocoumarins in the Apiaceae) with known defensive properties (Karban and Baldwin 1997, van der Putten et al. 2001, Bezemer et al. 2003, van Dam et al. 2003). As a result, I do not consider these findings to describe an idiosyncratic response that is unique to plants in the genus *Nicotiana*. Rather, the results from the tobacco system likely represent a phenomenon with broad implications for plant defense and linkages between above- and belowground communities.

Constitutive plant defenses in foliar vs. root tissues

Constitutive defenses, overall, were equally allocated between leaves and roots (Figure 2.4). This outcome is precisely what ODT would predict given that above- and belowground parts are considered to be similar in value and risk of attack from consumers (Zangerl and Bazzaz 1992). However, it should be noted that despite the remarkable similarity of overall secondary metabolite allocation between leaves and roots (52% and 48%, respectively) we found large amounts of variation in the dataset. The class of chemical measured partially explained this variation, with alkaloids exhibiting

higher foliar expression and glucosinolates demonstrating the opposite pattern with greater root expression.

I presume that these differences are not necessarily associated with biochemical constraints on where alkaloids/glucosinolates can be stored within the plant. Rather, I suspect that these differences may be driven by selective pressures imposed on certain plant groups that predominantly exploit alkaloids or glucosinolates as defenses. For instance, glucosinolates are largely restricted to plants in the Brassicaceae and, not surprisingly, the vast majority of studies in our dataset that quantified leaf and root glucosinolates comprised plants in this family. Consequently, the observation that roots contained higher concentrations of glucosinolates than leaves may simply reflect the fact that brassicaceous plants evolved in an environment that promoted this allocation pattern. This would be expected if root herbivores were relatively more common or damaging than foliar herbivores (greater risk) or if soil resources (e.g., water and nutrients) were more limiting than light availability (greater value) (Zangerl and Bazzaz 1992).

Herbivore-induced plant defenses in foliar vs. root tissues

If induced defenses to above- and belowground herbivores are allocated primarily on the basis of risk from future attack, then plants should induce stronger responses in the tissue type that is damaged compared with undamaged tissues (i.e., leaves should be more responsive than roots to foliar herbivores). In support of this prediction, I found that the magnitude of induction to aboveground herbivory was >2x greater in leaves than roots (Figure 2.5A). However, plant responses to belowground herbivory did not follow this pattern. Responses elicited in roots were equally expressed in foliar and root tissues

(Figure 2.5B). Thus, induced changes to leaf- and root-feeders are asymmetrical whereby root herbivory has systemic effects that propagate throughout the plant and leaf herbivory results in more localized changes in secondary plant chemistry. The ecological ramifications of these findings are that root herbivores may have stronger plant-mediated effects on foliar herbivores than the reverse situation. This asymmetry may be amplified further by the timing of attack to above- and belowground tissues with roots exposed to consumers well in advance of leaf expansion for many plant species (Bezemer and van Dam 2005). Yet the limited data on reciprocal plant-mediated interactions linking foliar and root herbivores do not entirely support the supposition that root-feeders are competitively dominant over foliar-feeders (Denno et al. 1995, van Dam et al. 2003, Denno and Kaplan 2007).

It remains unclear why such a discrepancy occurs in the systemic nature of plant responses to root and shoot herbivores. Clearly roots have the capacity to respond to herbivory as indicated by their strong induction to root herbivores (Figure 2.5B). One possibility might involve inherent differences in how plants translocate materials between above- and belowground tissues. Although bidirectional movement of solutes through the vascular system has been documented, materials, in general, move upward from the roots through the xylem and downward from the shoots in the phloem (Hopkins and Hüner 2004). For example, in the inducible nicotine system of tobacco, defoliation is thought to elicit defense signals that move from damaged leaf to roots via the phloem, triggering an increase in nicotine production which is translocated from biosynthetic sites in the roots to aboveground foliage via the xylem (Baldwin 1989, Baldwin et al. 1994, Zhang and Baldwin 1997). Therefore, differential translocation of defense signals/compounds in

vascular tissues may place physiological constraints on whole-plant induction (Davis et al. 1991, Shulaev et al. 1995, Arnold and Schultz 2002). In a similar manner, vascular architecture is known to strongly influence the spatial distribution of induced secondary compounds in plant leaves (Orians et al. 2000, Schittko and Baldwin 2003, Viswanathan and Thaler 2004).

Despite the apparent lack of symmetry in the magnitude of cross-system induction, herbivory elicited higher levels of secondary chemicals independent of which tissue was induced or where the response was measured [Figure 2.5; none of the error bars (95% CI) overlap zero]. The mere fact that such putative defenses were elevated at all in undamaged tissue is puzzling and seems to be sub-optimal from the plant's perspective (Wäckers and Bezemer 2003). Yet it is clear that this phenomenon occurs in an above/belowground context, as well as between other combinations of spatially distinct plant tissues (e.g., vegetative and reproductive parts; Euler and Baldwin 1996, McCall and Karban 2006, Adler et al. 2006). Below I outline a series of hypotheses that attempt to explain the paradox of cross-system induction (also see Wäckers and Bezemer 2003).

(1) Inducible secondary chemicals serve alternative functions

The function of secondary plant chemicals has long been debated (Fraenkel 1959, Ehrlich and Raven 1964, Levin 1971, Whittaker and Feeny 1971, Seigler and Price 1976, Jones 1979), but most ecologists now consider them to serve a defensive role involving interactions with herbivores. It has also become apparent that secondary chemicals (e.g., phenolics) are multi-functional in nature and thus have likely evolved under diffuse

selective pressures imposed by numerous biotic and abiotic challenges (Berenbaum 1995, Matsuki 1996, Seigler 1996). Although ODT predicts heterogeneity in plant defenses entirely on the basis of herbivore deterrence, this may underestimate the diversity of threats encountered by plants (Coleman and Jones 1991). Thus, it is difficult to reject the possibility that systemically induced changes in plant chemistry are not always allocated for the sole purpose of defense against herbivory.

(2) Physiological constraints on defense expression

An implicit assumption of ODT is that plants are unconstrained in how they distribute defenses. In other words, any allocation pattern is possible presuming that it minimizes costs and maximizes the efficacy of defenses. Yet detailed studies of intra-plant variation in induced aboveground defenses clearly demonstrate that expression of secondary chemicals are often mediated by vascular connections linking damaged with undamaged leaves (Davis et al. 1991, Shulaev et al. 1995, Orians et al. 2000, Arnold and Schultz 2002, Schittko and Baldwin 2003, Viswanathan and Thaler 2004). As a result, allocation must be viewed within the constraints imposed by plant physiology, which is not always consistent with the ideal strategy for herbivore deterrence. We know little about potential physiological constraints that shape patterns of induction between leaves and roots, but such constraints may partially explain why plants protect leaves when roots are attacked. Despite this possibility, empirical evidence increasingly provides support for the ODT principle that defenses are costly to plants (Zangerl and Bazzaz 1992, Purrington 2000, Heil and Baldwin 2002, Strauss et al. 2002). As a result, I presume that

selective pressures imposed from these costs would reduce inefficient and wasteful use of resources, making the documented cases of cross-system induction all the more puzzling.

(3) Synergistic effects of multiple enemies on plant fitness

Many plants have evolved the ability to tolerate herbivore damage (van der Meijden et al. 1988, Strauss and Agrawal 1999). However, herbivory by one consumer may alter the level of tolerance expressed to a 2nd herbivore, resulting in non-additive effects of multiple enemies on plant fitness (Strauss and Irwin 2004). For instance, several studies have found that the combined impact of 2 herbivores on plant performance is greater than the sum of each herbivore occurring individually (i.e., synergism – Strauss 1991, Pilson 1996, Wise and Sacchi 1996, Hufbauer and Root 2002, Eubanks et al. 2005). Under these circumstances it might be optimal to protect all parts following herbivory regardless of where the initial damage occurs, thus avoiding the more severe fitness penalties incurred by additional damage.

(4) Anticipation of future attack

Foliar defenses induced from root herbivory may be an adaptive response by the plant in anticipation of future aboveground attack. The logic underlying this hypothesis is based on the fact that several root herbivores implicated in cross-system induction are holometabolous insects that undergo ontogenetic shifts whereby larvae consume roots and adults subsequently emerge aboveground to attack leaves. Therefore, plants may preempt foliar herbivory by inducing aboveground defenses early and circumvent the time lag associated with inducing defenses after leaves are already damaged. This

possibility is problematic in that many foliar and root herbivores are restricted to feeding on either above- or belowground tissues, but not both (Brown and Gange 1990, Coleman et al. 2004). Furthermore, these types of consumers can nonetheless elicit cross-system induction. For example, we found higher concentrations of foliar phenolics and terpenoids in response to nematodes that are obligate root-feeders (Figure 3).

(5) Secondary invaders that act systemically

Plants are subject to attack by a diverse array of microorganisms (e.g., bacteria, fungi) that oftentimes can not infiltrate the peripheral defenses of healthy, undamaged plants (Agrios 2005). Herbivores, however, may act as “trojan horses” by opening wounds and thus facilitating entry by secondary invaders (Karban and Baldwin 1997). Phytoparasitic nematodes, for example, penetrate roots to access feeding sites and, in the process, create openings that are exploited by pathogens (Webster 1985, Sikora and Carter 1987, Abawi and Chen 1998). In many instances these secondary effects of nematodes via pathogens have a far greater impact on plant performance than the direct effects of nematode herbivory. If such pathogenic microorganisms are disseminated throughout the plant then it would be misleading to expect that defenses are only induced at the site of entry. For instance, the fungal pathogen *Sclerospora graminicola* infects root tissue, but most of the pathogen is subsequently localized in the shoot region (Sharada et al. 1995). Therefore, when assessing optimal defenses of plants to herbivory, one must consider the possibility that the response measured may not necessarily be elicited by the herbivore.

Conclusions

The importance of root herbivory is increasingly recognized in ecological studies, yet root herbivores remain minor contributors to our overall understanding of plant-herbivore interactions (van der Putten et al. 2001 van Dam et al. 2003, Bezemer and van Dam 2005, Stevens and Jones 2006, Preisser et al. 2007). Given that plant defenses often span above- and belowground systems, they represent a potentially important link that connects root herbivores with the foliar-based community (and vice versa). This is particularly true in light of induced defenses that are systemically expressed. Moreover, identifying potential similarities and differences in how plants interact with consumers of leaves and roots is instructive in assessing how ecological theory that has largely been developed aboveground (i.e., ODT) corresponds with interactions occurring belowground. Exploring these issues is timely given the recent and continuing interest among ecologists of interconnections between spatially separated communities (Wardle 2002, Wardle et al. 2004).

Table 2.1. The effects of root herbivory (nematodes), shoot herbivory (generalist and specialist caterpillars), and their interaction on above- and belowground secondary chemistry. Significant effects ($P < 0.05$) are highlighted in bold font.

Tissue	Compound	<i>Meloidogyne incognita</i> (root-galling nematode)			<i>Manduca sexta</i> (specialist caterpillar)			<i>Trichoplusia ni</i> (generalist caterpillar)		
		df	F	<i>P</i>	df	F	<i>P</i>	df	F	<i>P</i>
Leaf	MANOVA	6, 71	20.74	<0.0001	6, 71	6.73	<0.0001	6, 71	32.11	<0.0001
	Nicotine	1, 63	79.92	<0.0001	1, 63	5.98	0.0173	1, 63	20.05	<0.0001
	Non-nicotine alkaloids	1, 63	24.31	<0.0001	1, 63	5.07	0.0279	1, 63	8.48	0.0050
	Chlorogenic acid	1, 63	4.57	0.0365	1, 63	1.54	0.2192	1, 63	0.38	0.5373
	Rutin	1, 63	11.83	0.0010	1, 63	10.10	0.0023	1, 63	8.70	0.0045
	Phenolic-2	1, 63	7.20	0.0093	1, 63	0.37	0.5469	1, 63	1.570	0.2145
	Diterpene glycosides	1, 63	6.22	0.0153	1, 63	20.46	<0.0001	1, 63	153.62	<0.0001
Root	MANOVA	6, 68	10.74	<0.0001	6, 68	1.22	0.3061	6, 68	1.21	0.3119
	Nicotine	1, 60	20.10	<0.0001	1, 60	0.23	0.6301	1, 60	0.14	0.7082
	Non-nicotine alkaloids	1, 60	14.80	0.0003	1, 60	0.01	0.9304	1, 60	0.07	0.7910
	Chlorogenic acid	1, 60	0.00	0.9937	1, 60	0.28	0.6006	1, 60	0.84	0.3618
	Rutin	1, 60	1.61	0.2099	1, 60	0.60	0.4400	1, 60	1.72	0.1945
	Phenolic-2	1, 60	6.44	0.0138	1, 60	0.99	0.3230	1, 60	0.59	0.4441
	Phenolic-1	1, 60	8.25	0.0056	1, 60	0.16	0.6950	1, 60	0.06	0.8144
Gall	MANOVA	6, 66	220.67	<0.0001	6, 66	0.42	0.8605	6, 66	1.48	0.1976
	Nicotine	1, 58	79.14	<0.0001	1, 58	0.14	0.7072	1, 58	0.00	0.9893
	Non-nicotine alkaloids	1, 58	196.64	<0.0001	1, 58	0.20	0.6526	1, 58	0.69	0.4110
	Chlorogenic acid	1, 58	7.44	0.0084	1, 58	1.44	0.2343	1, 58	4.41	0.0401
	Rutin	1, 58	1353.55	<0.0001	1, 58	0.00	0.9807	1, 58	0.04	0.8353
	Phenolic-2	1, 58	650.91	<0.0001	1, 58	0.00	0.9999	1, 58	0.12	0.7291
	Phenolic-1	1, 58	199.43	<0.0001	1, 58	1.37	0.2469	1, 58	2.82	0.0985

Table 2.1 (continued)

Tissue	Compound	<i>Manduca sexta</i>			<i>Trichoplusia ni</i>		
		x <i>Meloidogyne incognita</i>			x <i>Meloidogyne incognita</i>		
		df	F	P	df	F	P
Leaf	MANOVA	6, 71	1.26	0.2877	6, 71	4.59	0.0005
	Nicotine	1, 63	2.61	0.1114	1, 63	7.35	0.0087
	Non-nicotine alkaloids	1, 63	0.94	0.3360	1, 63	1.55	0.2181
	Chlorogenic acid	1, 63	0.66	0.4184	1, 63	20.23	<0.0001
	Rutin	1, 63	0.84	0.3636	1, 63	15.37	0.0002
	Phenolic-2	1, 63	0.46	0.4987	1, 63	0.61	0.4377
	Diterpene glycosides	1, 63	2.33	0.1320	1, 63	0.01	0.9048
Root	MANOVA	6, 68	0.86	0.5281	6, 68	2.27	0.0466
	Nicotine	1, 60	0.58	0.4493	1, 60	0.16	0.6908
	Non-nicotine alkaloids	1, 60	0.03	0.8598	1, 60	1.10	0.2989
	Chlorogenic acid	1, 60	0.20	0.6566	1, 60	0.00	0.9452
	Rutin	1, 60	0.24	0.6251	1, 60	1.61	0.2089
	Phenolic-2	1, 60	0.00	0.9951	1, 60	1.45	0.2340
	Phenolic-1	1, 60	1.47	0.2294	1, 60	4.92	0.0303
Gall	MANOVA	6, 66	1.47	0.2029	6, 66	1.67	0.1432
	Nicotine	1, 58	0.30	0.5843	1, 58	0.81	0.3725
	Non-nicotine alkaloids	1, 58	0.66	0.4183	1, 58	0.01	0.9061
	Chlorogenic acid	1, 58	1.62	0.2088	1, 58	2.43	0.1242
	Rutin	1, 58	0.33	0.5669	1, 58	0.00	0.9541
	Phenolic-2	1, 58	1.32	0.2547	1, 58	0.02	0.8826
	Phenolic-1	1, 58	0.16	0.6887	1, 58	0.48	0.4896

Figure Legends

Figure 2.1. The impact of aboveground herbivory (specialist caterpillar, *M. sexta*, and generalist caterpillar, *T. ni*) and belowground herbivory (nematode, *M. incognita*) on secondary chemistry of tobacco roots, including (A) nicotine, (B) non-nicotine alkaloids, (C) chlorogenic acid, (D) rutin, (E) phenolic-1, and (F) phenolic-2 (means + SE). fw = fresh weight, pa = peak area.

Figure 2.2. The impact of aboveground (specialist caterpillar, *M. sexta*, and generalist caterpillar, *T. ni*) and belowground herbivory (nematode, *M. incognita*) on secondary chemistry of tobacco leaves, including (A) nicotine, (B) non-nicotine alkaloids, (C) chlorogenic acid, (D) rutin, (E) phenolic-2, and (F) diterpene glycosides (means + SE). fw = fresh weight, pa = peak area.

Figure 2.3. Effect sizes comparing the impact of nematode root herbivory on tobacco foliar defenses that are synthesized either above- or belowground. Numbers above or below error bars represent the number of observations per class. Error bars = 95% confidence intervals.

Figure 2.4. Comparison of constitutive plant defenses expressed in foliar vs. root tissues. Data were obtained from a literature review of studies quantifying the concentrations of secondary plant chemicals above- and belowground. Error bars = 95% confidence intervals.

Figure 2.5. Induced changes in above- and belowground plant defenses for responses that are elicited in (A) leaves and (B) roots. A meta-analysis was conducted using published studies that quantified induced plant defenses in an above/belowground context. Numbers above error bars represent the number of observations per class. Error bars = 95% confidence intervals.

Figure 2.1

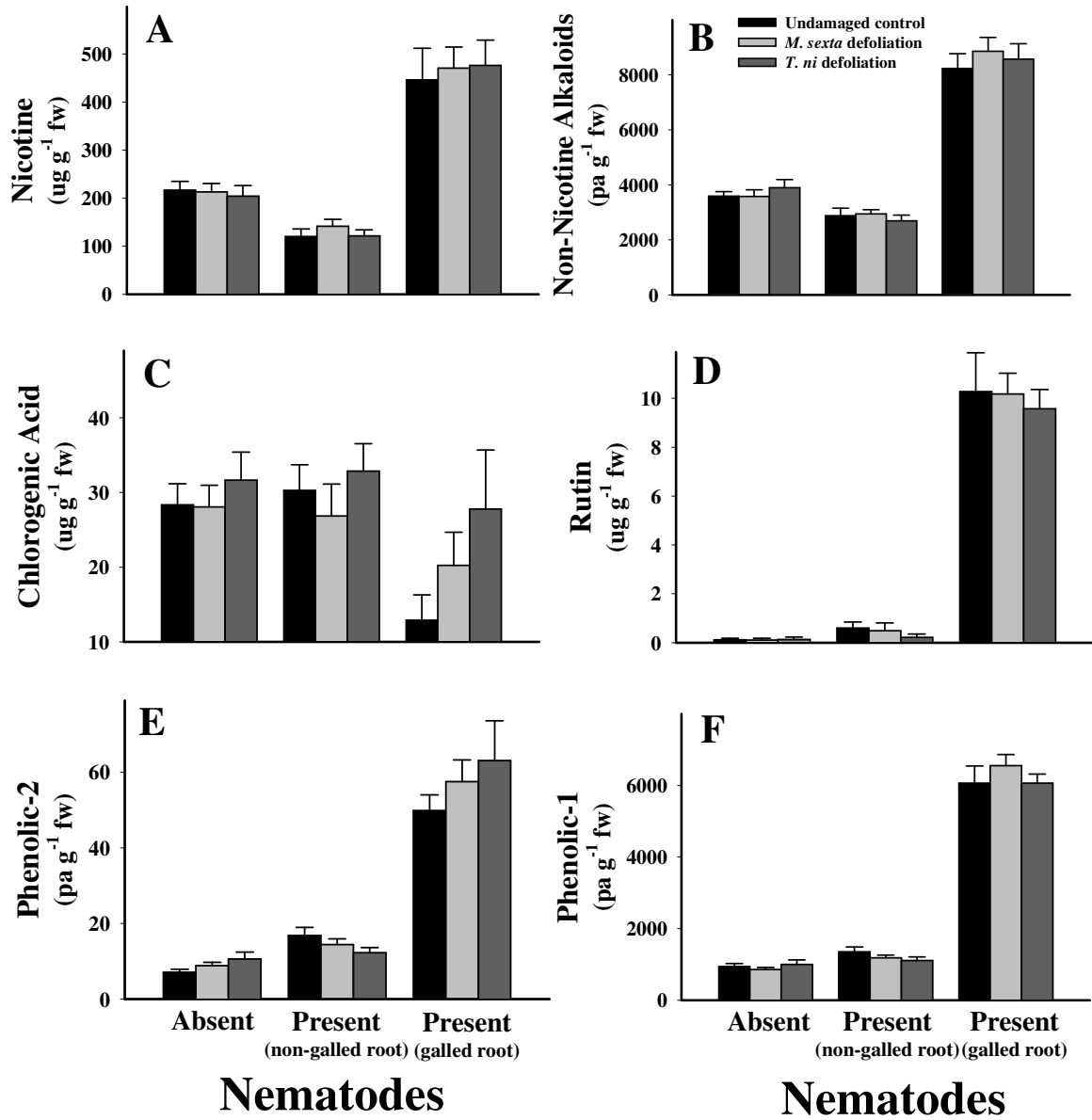


Figure 2.2

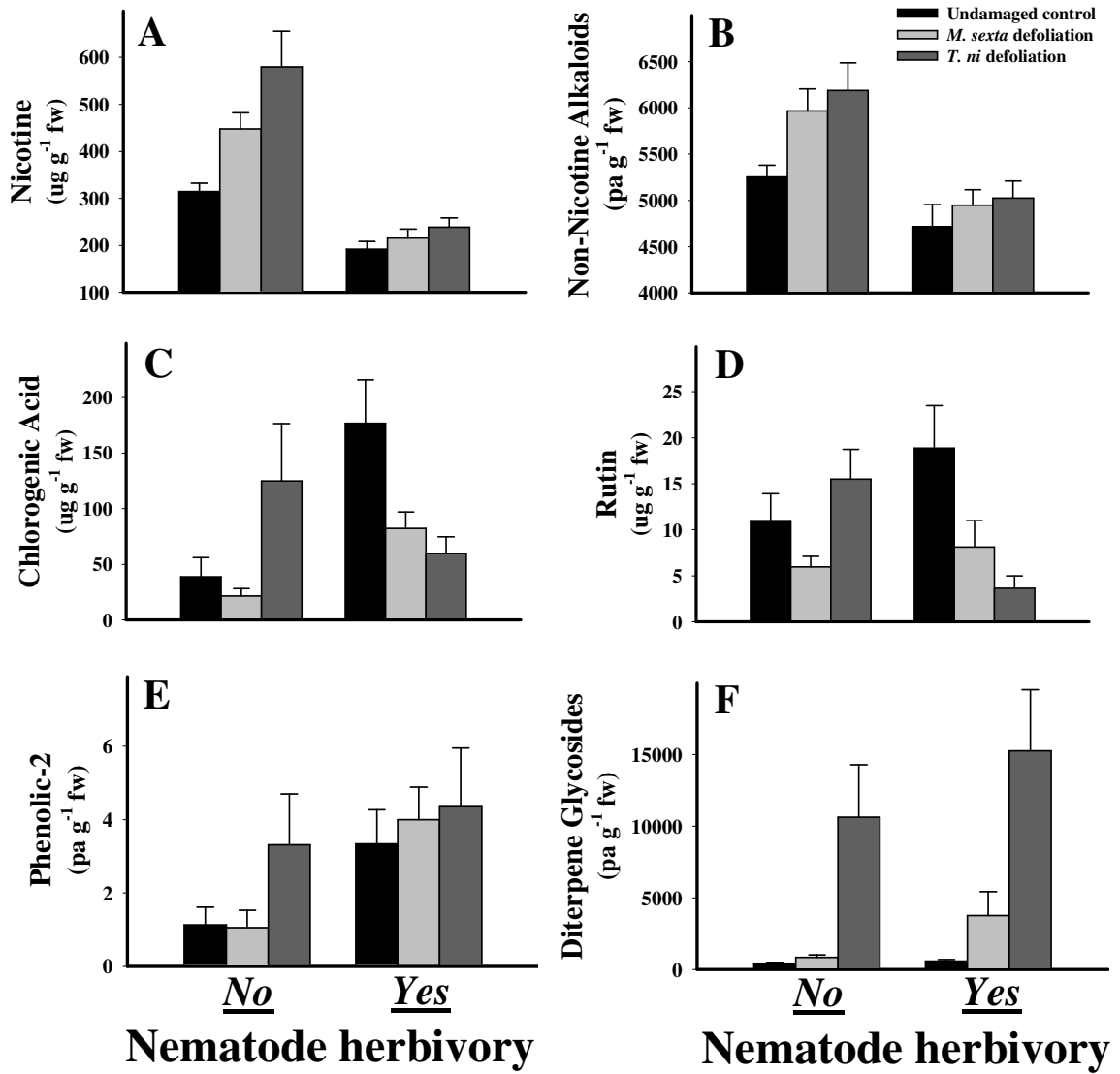


Figure 2.3

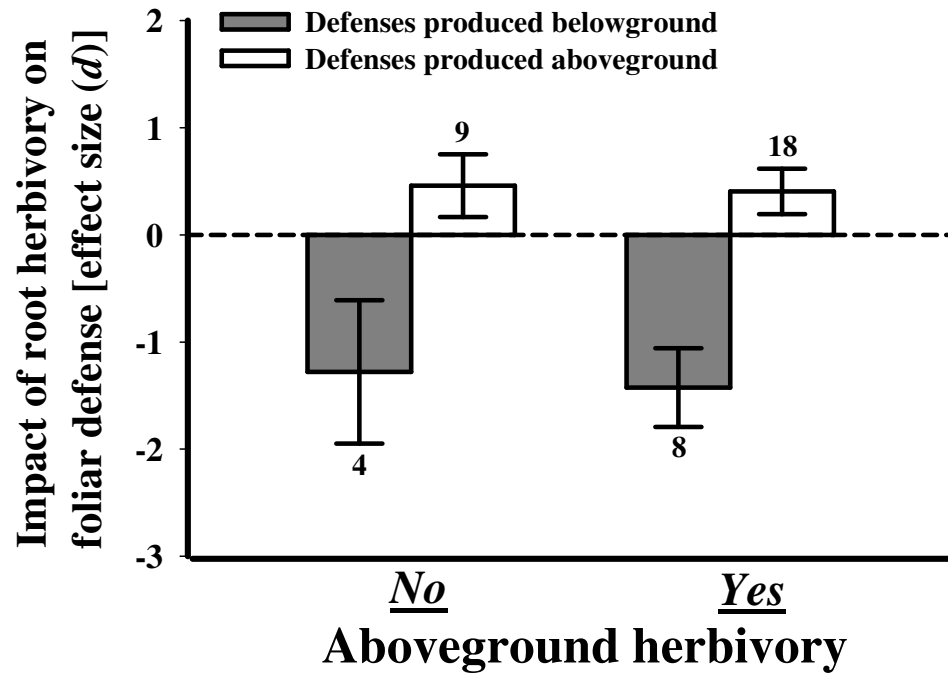


Figure 2.4

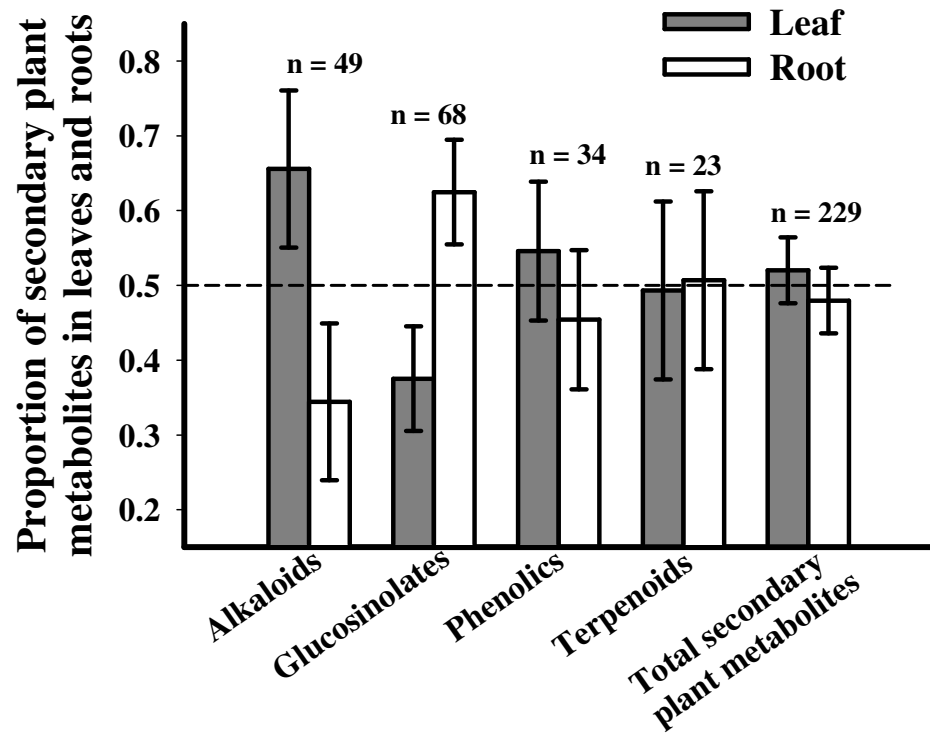
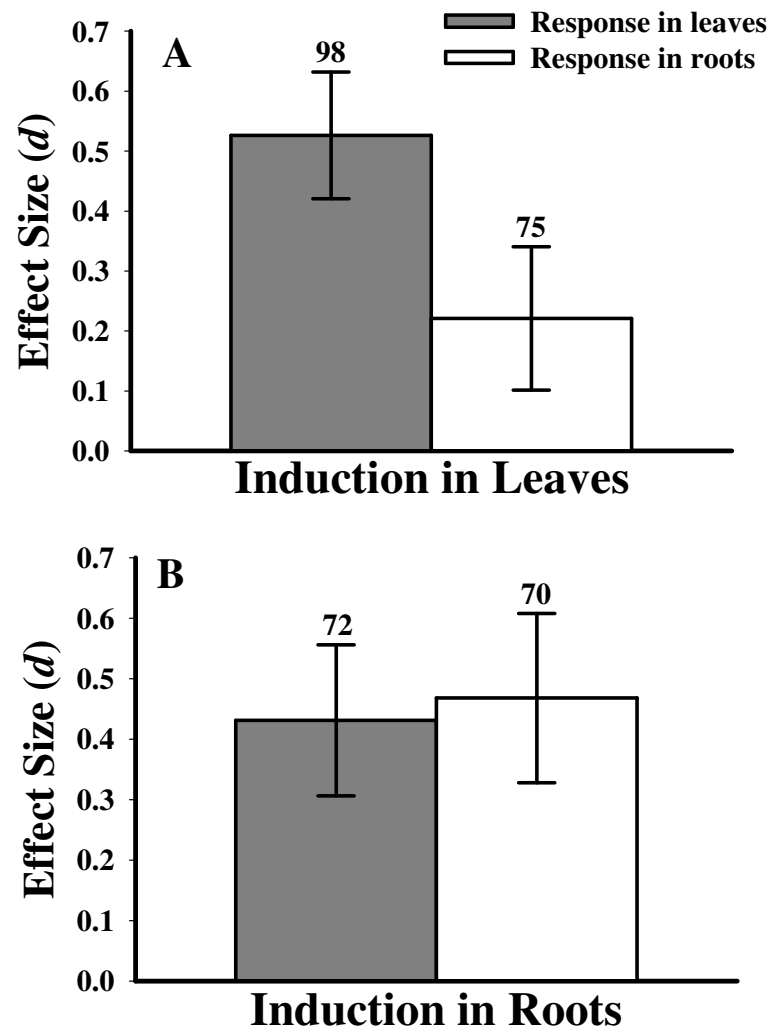


Figure 2.5



CHAPTER 3: Effects of plant vascular architecture on above-belowground induced responses to foliar and root herbivores on *Nicotiana tabacum*

Abstract

Herbivores induce systemic changes in plant traits and the strength of these induced responses is often associated with the degree of vascular connectivity linking damaged and undamaged tissues. Although this phenomenon is known to occur aboveground in leaves, it is unknown if leaf-root induction similarly follows the vascular architecture of plants. To test for this possibility I manipulated foliar and root herbivory (the leaf-chewing insect, *Spodoptera exigua*, and the root-galling nematode, *Meloidogyne incognita*) on tobacco (*Nicotiana tabacum*) and measured subsequent changes in secondary chemistry (alkaloids and phenolics) in leaves and roots that were orthostichous (vertically aligned) and non-orthostichous (opposite) from the herbivore-damaged tissue. Aboveground caterpillar herbivory elicited stronger secondary chemical responses in orthostichous compared with non-orthostichous leaves and roots. Belowground nematode herbivory, however, did not affect the secondary chemistry of tobacco leaves. Thus, plant vascular architecture mediates the magnitude of systemic induction in roots as well as in leaves, with overall stronger responses in tissues that are more closely aligned. As a result, herbivores that co-occur on the same sector of plant (both above- and belowground) may be more likely to affect one another via induced responses than herbivores that occur on plant tissues sharing fewer resources.

Introduction

Herbivore-induced plant responses are often systemic in nature, but their expression is not uniformly distributed across undamaged tissues (Karban and Baldwin 1997, Orians 2005). Instead, herbivore feeding typically elicits a response that varies quantitatively among plant parts, with some leaves responding strongly and others not at all (Stout et al. 1996, Viswanathan and Thaler 2004, Shelton 2005). Such within-plant variation in the magnitude of induction generates fine-scale patterns of plant defense, and this heterogeneity has important ecological implications for plant-herbivore interactions at large (Denno and McClure 1983, Hunter and Price 1992, Orians et al. 2002).

The most widely-cited explanation for leaf-to-leaf variation in the strength of systemic induction is that leaves are differentially interconnected with other leaves via the plant's vascular system (Orians 2005). As a result, many phytochemicals, including the signals and resources required for launching plant defense responses, are restricted in their translocation patterns (Davis et al. 1991, Arnold and Schultz 2002, Arnold et al. 2004). Although it has long been known that sectorial transport influences the allocation of carbon (i.e., sugars) between plant sources and sinks (Watson and Casper 1984, Sprugel et al. 1991, Vuorisalo and Hutchings 1996), the impact of plant sectoriality on induced responses to herbivory has only recently been described (Jones et al. 1993, Mutikainen et al. 1996, Orians et al. 2000, Schittko and Baldwin 2003, Viswanathan and Thaler 2004). In these cases, leaves that share the strongest vascular connections (i.e., orthostichous pairs) also tend to transmit the strongest induced responses (Orians 2005; but see Frost et al. 2007).

However, the consequences of plant vascular architecture for induction have thus far only been emphasized in responses that are both elicited and expressed in foliar

tissues. Recent studies demonstrate that induced responses in fact span the above- and belowground systems (van Dam et al. 2003, Bezemer and van Dam 2005, van Dam and Bezemer 2006). Thus, root herbivory can induce changes in the secondary chemistry of leaves (Bezemer et al. 2004, Soler et al. 2005, van Dam et al. 2005, van Dam and Raaijmakers 2006, Kaplan et al. in press), and leaf herbivory can similarly induce changes in root chemistry (Soler et al. 2007). Because resource sharing between leaves and roots is known to be sectorial in certain plants (Orians et al. 2004, Bledsoe and Orians 2006, Zanne et al. 2006), vascular architecture may influence aboveground-belowground induction patterns in a manner analogous to orthostichous leaves. Yet, to my knowledge, this possibility has never been tested. Therefore, I experimentally manipulated foliar and root herbivory on tobacco (*Nicotiana tabacum*) using a split-root design, and quantified the effects of plant vascular architecture on above-belowground induced chemical responses. If leaf-root induction is indeed sectorial, as is often the case with leaves, this has important ecological relevance towards understanding the causes of heterogeneity in plant defense traits and their extended effects on consumers.

Methods

I tested the impact of plant sectoriality on herbivore-induced secondary chemical responses in leaves and roots. Tobacco plants (var. MD 609) were propagated from seed in a greenhouse and seedlings were transplanted after nine weeks of growth to pots containing a sterilized growing medium [50% sand, 50% potting mix (SunGro LC1 and professional blend; sphagnum peat moss, bark, perlite, vermiculite, and clay)]. Plants were supplemented with nutrients weekly by applying a soluble fertilizer (20:10:20

NPK). A split-root technique was used whereby the roots of all seedlings were divided and each of the two halves was transplanted into separate but adjacent 2-gallon pots. Thus, each plant possessed a single root system divided equally between two independent growth environments.

Experiment 1 – Aboveground herbivory

Foliar herbivory was manipulated by applying beet armyworm caterpillars, *Spodoptera exigua*, to selected leaves for defoliation. Caterpillars were obtained from a biological supply company (Benzon Research, Inc., Carlisle, PA) and reared on artificial diet until they reached the 3rd instar. Fine-mesh sleeve cages were placed over leaves and fastened to the petiole using a twist-tie to ensure that caterpillars only damaged the leaf assigned to the foliar herbivory treatment.

Thirty split-root plants were used and half of these were randomly assigned to caterpillar defoliation (n=15) while the other half served as undamaged controls. Damage regimes were initiated 21 days after seedlings were transplanted into pots and thus remained in the rosette stage of growth. The first fully-expanded leaf that was orthostichous (i.e., vertically aligned) with one of the two root sections (i.e., pots) was assigned to caterpillar damage. Three 3rd instar *S. exigua* larvae were placed in sleeve cages and remained for 72 hrs, during which time they removed 20-50% of the leaf area. Control plants also received sleeve cages, but no caterpillars were added. Forty-eight hrs later, and 5 d from the initiation of damage regimes, leaves and roots were harvested for secondary chemical analysis.

Two recently expanded sink leaves were collected from each plant, one leaf that was orthostichous with the caterpillar-damaged leaf and a second leaf that was opposite from the damaged leaf. Rosette-stage tobacco plants follow a 3/8 phyllotaxis whereby each new leaf emerges at a 135° angle from the previous leaf (Allard 1942, Jones et al. 1959, Schittko and Baldwin 2003). Therefore, the leaf that is 8 positions higher on the plant from the damaged leaf is vertically aligned. Additionally, root tissue was harvested from both pots in each plant for chemical analysis.

Tissue samples were immediately frozen in liquid nitrogen and secondary chemicals with known anti-herbivore properties were extracted and analyzed by HPLC (see Keinänen et al. 2001) on a reversed phase C18 column (Gemini C18, 150 x 4.6 mm; Phenomenex). Concentrations of identified alkaloids (nicotine) and phenolics (chlorogenic acid and rutin) were quantified using calibration curves prepared from commercially available standards. Concentrations of non-nicotine alkaloids, caffeoyl putrescine, and 7-methyl esculin were calculated from peak areas at 254 nm (for alkaloids) and 320 nm (for caffeoyl putrescine and 7-methyl esculin).

Experiment 2 – Belowground herbivory

Root herbivory was manipulated by inoculating tobacco roots with the nematode *Meloidogyne incognita*. This polyphagous species is a sedentary endoparasite that induces a gall at feeding sites (Trudgill and Block 2001). Nematode cultures originated from local agricultural fields and were reared on tobacco prior to use in experiments. Nematode eggs were harvested from the roots of heavily infested plants using a modified version of the Hussey and Barker (1973) extraction procedure. Galled roots were cut into

2 cm long segments, placed into a 250 ml flask containing 100 ml of a 0.6% NaOCl dilution, and subsequently poured through nested sieves (250- μ m-pore sieve on top of a 25- μ m-pore sieve). The resulting eggs were counted and applied to the roots of experimentally-inoculated seedlings at transplant. Each seedling assigned to the root herbivory treatment received ~100,000 *M. incognita* eggs. This density falls well within the range documented for *M. incognita*-infested tobacco fields (Barker and Lucas 1984) and also corresponds with inoculum levels used in prior studies on *M. incognita*-tobacco interactions (Hanounik and Osbourne 1975, 1977, Barker and Weeks 1991, Wheeler et al. 1991, Vovlas et al. 2004).

Thirty split-root plants were used and half of these were assigned to the nematode root herbivory treatment (n=15) while the other half acted as control plants. On nematode-treated plants, roots from only one of the two pots were inoculated with nematodes. Thus, in the root herbivory treatment only half of the root system was galled by nematodes and the other half was not. Twenty-one days after nematode addition plant leaves were harvested for secondary chemical analysis. Two recently expanded sink leaves were harvested from each plant, one leaf that was orthostichous (i.e., vertically aligned) with the pot containing nematode-inoculated roots and a second leaf that was opposite from the inoculated root section. On control plants, the two leaves were chosen to align with each of the two pots as in the nematode treatment. Secondary plant chemicals were quantified in leaf tissue samples as described above in Experiment 1.

Statistical Analyses

To assess the impact of plant vascular architecture on induced responses to foliar and root herbivory I used MANOVA (Proc glm; statistical analyses were performed using SAS, Version 9.1; SAS Institute, Inc., 2001), followed by univariate ANOVAs for each secondary chemical measured (Proc mixed). Separate analyses were performed to quantify the impact of aboveground caterpillar herbivory on orthostichous and non-orthostichous leaves and roots, as well as the effect of belowground nematode herbivory on orthostichous and non-orthostichous leaves. Spatial groupings of control and induced plants were considered blocks and treated as a random effect in the model. Data were transformed using square-root and log transformations as needed to meet assumptions of normality and homogeneity of variances.

To directly compare the overall magnitude of secondary chemical induction in orthostichous vs. non-orthostichous plant tissues I calculated effect sizes (Hedges' *d*) for the impact of caterpillar herbivory on leaf and root chemistry, and the effect of nematode herbivory on leaf chemistry (Rosenberg et al. 2000). Mixed-effects categorical models were then used to compare effect sizes in orthostichous and non-orthostichous plant tissues. For each group a mean effect size was calculated and reported with 95% bootstrap confidence intervals. Effect sizes greater than zero indicate that herbivory elicited an overall increase in the concentration of secondary plant chemicals. Between-group heterogeneity (Q_B) was tested against a χ^2 -distribution to determine if plant vascular architecture affected the magnitude of above- and belowground induction.

Results

Foliar herbivory elevated the secondary chemistry of tobacco leaves that were both orthostichous (Figs. 3.1A-E) and non-orthostichous (Figs. 3.1F-J) with the caterpillar-damaged leaf (Table 3.1A). However, the impact of phytochemical induction was more apparent in leaves sharing strong vascular connections. Four of the 5 secondary chemicals measured (nicotine, non-nicotine alkaloids, caffeoyl putrescine, and chlorogenic acid) were induced to higher levels in orthostichous leaves (Figs. 3.1A-D), compared with only 1 of 5 (nicotine) in non-orthostichous leaves (Fig. 3.1F).

Broadly similar results were found when comparing the effects of aboveground herbivory on root chemistry in vertically-aligned (Figs. 3.2A-E) vs. opposing (Figs. 3.2F-J) root sections (Table 3.1B). Caterpillar feeding elevated the belowground concentration of certain compounds (e.g., non-nicotine alkaloids; Fig. 3.2B) and there was a trend for herbivory to affect overall secondary chemical expression in orthostichous roots (MANOVA: $P = 0.075$). In contrast, aboveground induction clearly did not affect the chemistry of roots that were opposite the insect-defoliated leaf (Figs. 3.2F-J).

Nematode root herbivory had no impact on leaf chemistry in either of the two leaf tissue types (Table 3.1C), with non-significant effects of nematodes on secondary compounds in both orthostichous (Figs. 3.3A-E) and non-orthostichous (Figs. 3.3F-J) leaves.

The overall magnitude of phytochemical induction differed between orthostichous and non-orthostichous plant tissues for one of the three comparisons, i.e., the impact of caterpillar herbivory on leaf chemistry ($Q_B = 4.90$, $df = 1$, $P = 0.027$). In this case, the effect size for responses in the orthostichous leaf was >2x as large as in the non-orthostichous leaf (Fig. 3.4A). The difference in root responses to foliar herbivory was

near significance ($Q_B = 2.90$, $df = 1$, $P = 0.089$), with moderately strong responses in vertically-aligned roots (effect size = 0.45) and virtually no response in opposing root sections (effect size = 0.02; Fig. 3.4B). However, aboveground responses to root herbivory were equally weak in both leaf tissue types ($Q_B = 0.03$, $df = 1$, $P = 0.864$; Fig. 3.4C).

Discussions

Vascular architecture is known to mediate herbivore-induced plant responses that are both elicited and expressed in leaves, but it is unknown if vascular connections similarly shape patterns of induction linking leaves with roots. In agreement with previously published accounts (e.g., Orians et al. 2000, Schittko and Baldwin 2003, Viswanathan and Thaler 2004), I found that the magnitude of phytochemical induction to foliar herbivory was most pronounced in leaves sharing strong vascular connections with the caterpillar-damaged leaf (Figs. 3.1 and 3.4A). However, I also document the hitherto unreported and novel finding that vascular architecture mediates the effects of foliar herbivory on root chemistry, with stronger induction in roots that are vertically-aligned with insect-defoliated leaves (Figs. 3.2 and 3.4B). Thus, responses to aboveground insect herbivory in tobacco are not uniformly distributed within the plant. Rather, responses are sectorized whereby closely-aligned tissues that share a disproportionate quantity of resources (e.g., photosynthates) also transmit the strongest inducible responses.

Because studies that measure the impact of leaf herbivory on root chemistry do not account for vascular architecture, whereas studies on aboveground induced responses often do, the magnitude of root responses to foliar induction may be underestimated in

the literature. For example, in a recently published meta-analysis that merged data from multiple independent studies, evidence was found that foliar induction elicits a relatively weak response in root tissue (Kaplan et al. in press). This outcome may in part be driven by the lack of control over leaf-root connectivity in prior studies, and future investigations on above-belowground induction should consider plant sectoriality when designing experiments. However, leaf responses to foliar herbivory were nevertheless stronger than root responses in the present study, despite controlling for vascular architecture in both cases (leaf-leaf effect size = +0.982, leaf-root effect size = +0.446).

Surprisingly, root herbivory did not alter the secondary chemistry of tobacco leaves (Fig. 3.3), making it difficult to evaluate the consequences of vascular architecture (Fig. 3.4C). This outcome was unexpected because in prior experiments strong foliar responses to nematode (*M. incognita*) root herbivory were documented using the same tobacco system and under similar environmental and ecological conditions (Kaplan et al. in press). Thus, nematode herbivory in only half of the root system appears to elicit very different aboveground responses when compared with plants experiencing wide-spread herbivory distributed throughout the entire root system. The quantity of tissue damaged is known to affect the strength of herbivore-induced responses in leaves (see Table 4.5 in Karban and Baldwin 1997). Therefore, lower levels of root herbivory may lead to lower levels of aboveground induction. Because nematodes damage alkaloid biosynthetic sites and interfere with foliar expression (Hanounik and Osborne 1977, Barker and Weeks 1991, Kaplan et al. in press), another potential explanation is that plants compensate for root damage on one side of the plant by increasing alkaloid production on the other undamaged side.

Conclusions

Foliar and root herbivores are often patchily distributed on plant leaves and roots (Denno and McClure 1983, Ettema and Wardle 2002). As a result, their systemic effects on plant defense traits are also likely to be heterogeneous. Given the recent emphasis on linkages between above- and belowground biota (Wardle et al. 2002, 2004), these findings provide new and important mechanistic insight into the ecological circumstances in which foliar and root herbivores affect both plant chemistry and potentially other co-occurring herbivores.

Table 3.1. The effects of aboveground and belowground herbivory on the expression of secondary plant chemicals in orthostichous and non-orthostichous leaves and roots of tobacco. Significant effects ($P < 0.05$) are bolded for emphasis.

Herbivory	Tissue	Compound	Orthostichous			Non-orthostichous		
			df	F	<i>P</i>	df	F	<i>P</i>
(A) Caterpillar Aboveground	Leaf	MANOVA	5, 22	8.98	< 0.001	5, 24	3.02	0.030
		Nicotine	1, 12	19.43	< 0.001	1, 14	7.83	0.014
		Non-nicotine alkaloids	1, 12	13.65	0.003	1, 14	0.79	0.389
		Caffeoyl putrescine	1, 12	9.09	0.011	1, 14	0.99	0.336
		Chlorogenic acid	1, 12	7.19	0.020	1, 14	0.11	0.750
		Rutin	1, 12	2.32	0.154	1, 14	2.49	0.137
(B) Caterpillar Aboveground	Root	MANOVA	5, 23	2.33	0.075	5, 18	0.63	0.681
		Nicotine	1, 13	1.88	0.194	1, 10	0.07	0.791
		Non-nicotine alkaloids	1, 13	9.09	0.010	1, 10	0.00	0.979
		Caffeoyl putrescine	1, 13	2.64	0.128	1, 10	0.72	0.415
		Chlorogenic acid	1, 13	4.01	0.066	1, 10	0.09	0.765
		7-methyl esculin	1, 13	0.48	0.499	1, 10	4.33	0.064
(C) Nematode Belowground	Leaf	MANOVA	5, 23	0.89	0.505	5, 23	0.48	0.784
		Nicotine	1, 13	1.29	0.276	1, 13	0.71	0.416
		Non-nicotine alkaloids	1, 13	0.01	0.935	1, 13	0.09	0.769
		Caffeoyl putrescine	1, 13	0.01	0.910	1, 13	0.00	0.957
		Chlorogenic acid	1, 13	0.05	0.821	1, 13	0.55	0.470
		Rutin	1, 13	1.43	0.254	1, 13	0.27	0.609

Figure Legends

Figure 3.1. The effects of aboveground caterpillar herbivory on the expression of secondary plant chemicals in orthostichous (A-E) and non-orthostichous (F-J) tobacco leaves, including nicotine, non-nicotine alkaloids, caffeoyl putrescine, chlorogenic acid, and rutin (means \pm SE). An asterisk denotes significant differences between means ($P < 0.05$).

Figure 3.2. The effects of aboveground caterpillar herbivory on the expression of secondary plant chemicals in orthostichous (A-E) and non-orthostichous (F-J) tobacco roots, including nicotine, non-nicotine alkaloids, caffeoyl putrescine, chlorogenic acid, and 7-methyl esculin (means \pm SE). An asterisk denotes significant differences between means ($P < 0.05$).

Figure 3.3. The effects of belowground nematode herbivory on the expression of secondary plant chemicals in orthostichous (A-E) and non-orthostichous (F-J) tobacco leaves, including nicotine, non-nicotine alkaloids, caffeoyl putrescine, chlorogenic acid, and rutin (means \pm SE). An asterisk denotes significant differences between means ($P < 0.05$).

Figure 3.4. The magnitude of herbivore-induced responses in orthostichous vs. non-orthostichous plant tissues, including (A) leaf responses to foliar herbivory, (B) root responses to foliar herbivory, and (C) leaf responses to root herbivory (means \pm 95% bootstrap confidence intervals). Effect sizes (Hedges' d) were calculated to summarize

the cumulative secondary chemical response to herbivory in leaves and roots. An asterisk denotes significant differences between means ($P < 0.05$). n.s. = non-significant

Figure 3.1

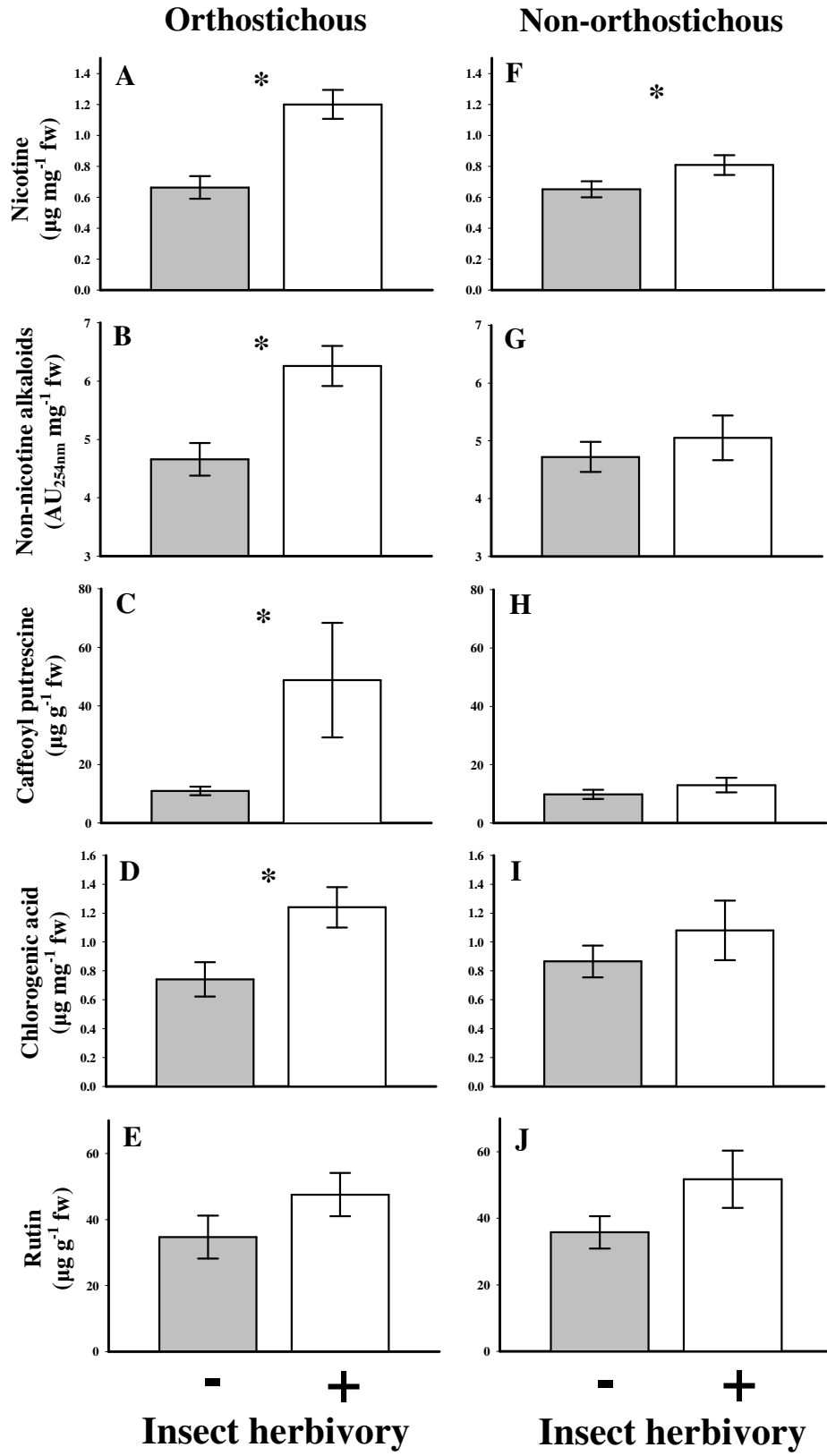


Figure 3.2

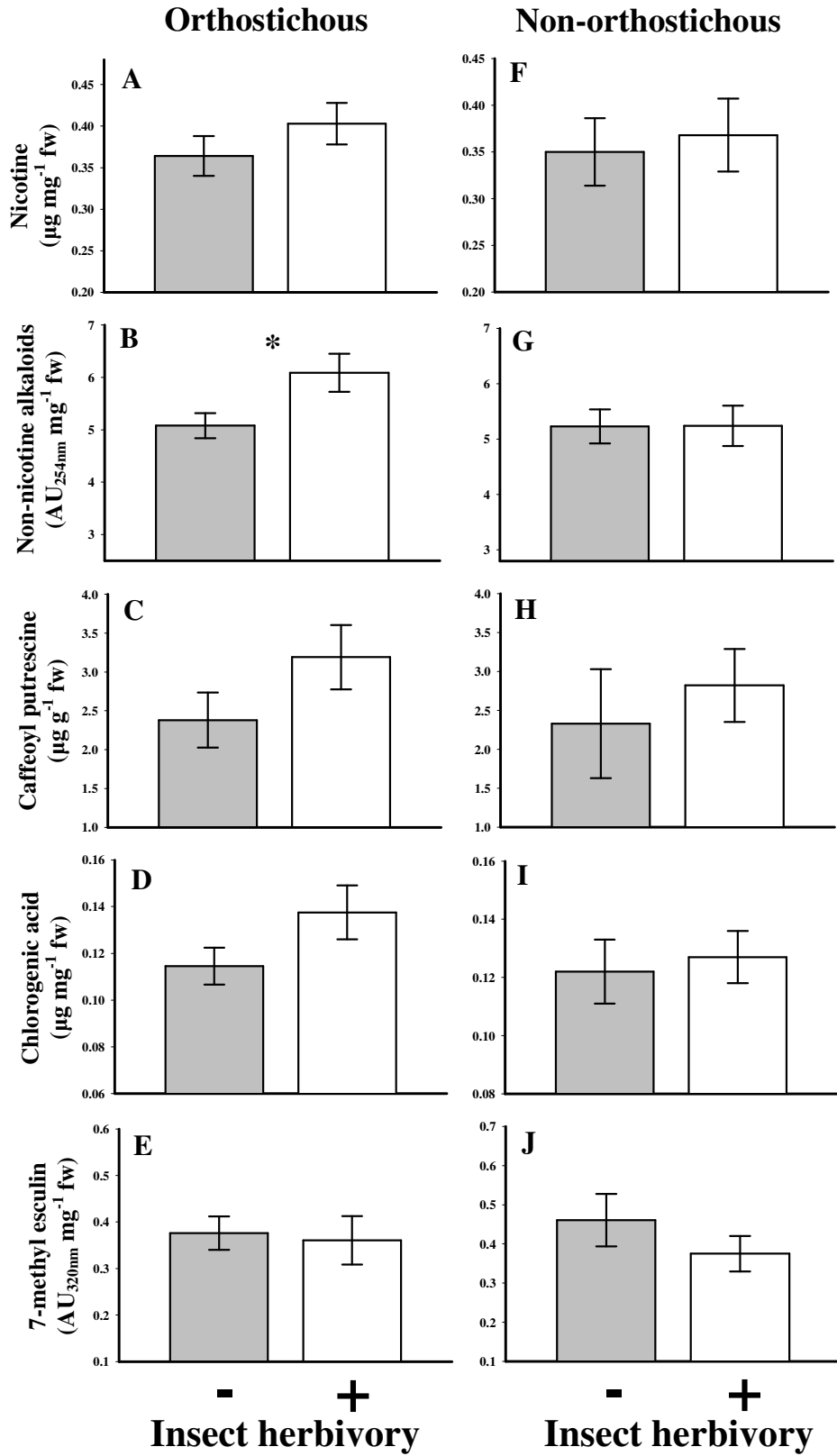


Figure 3.3

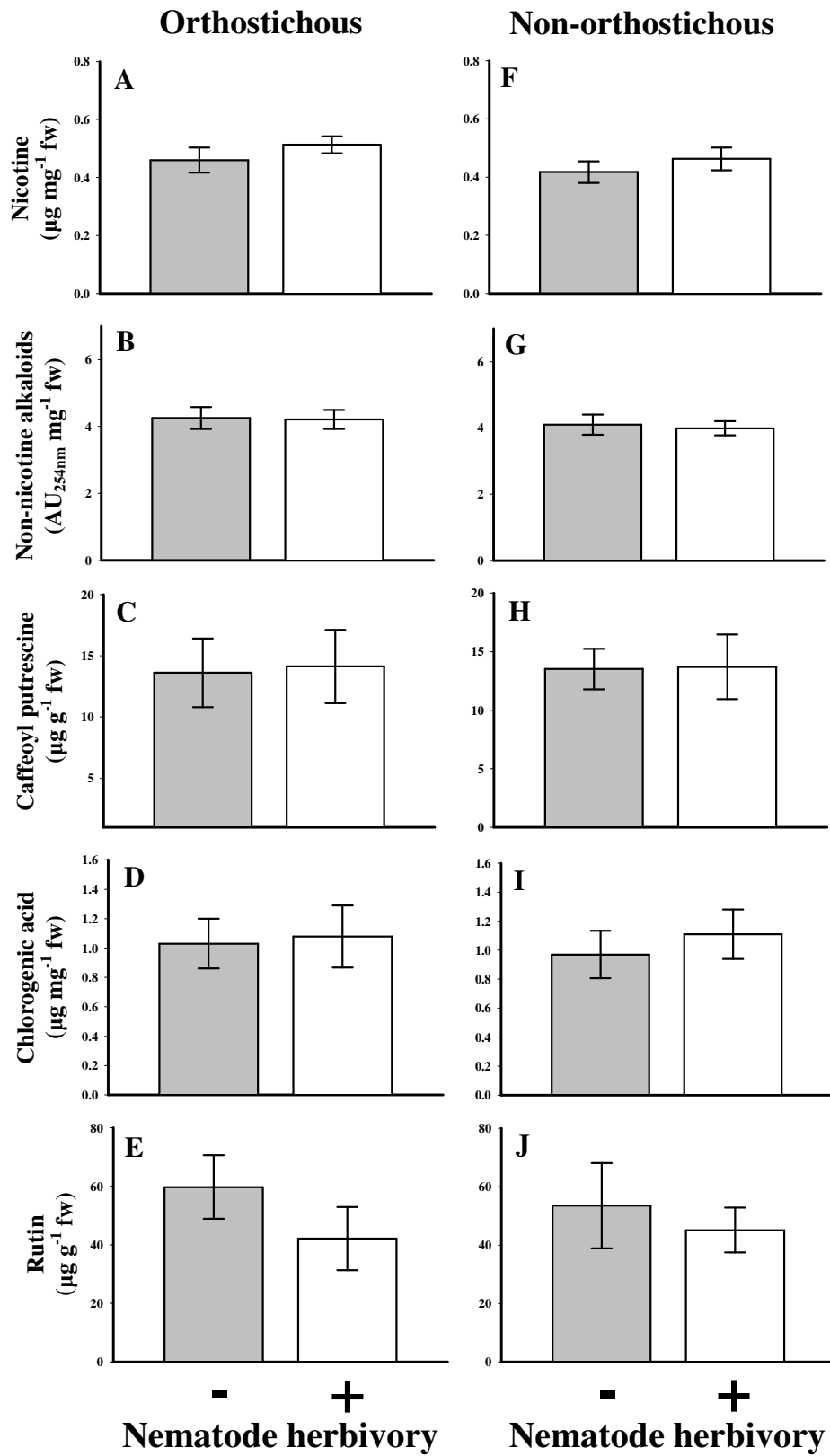
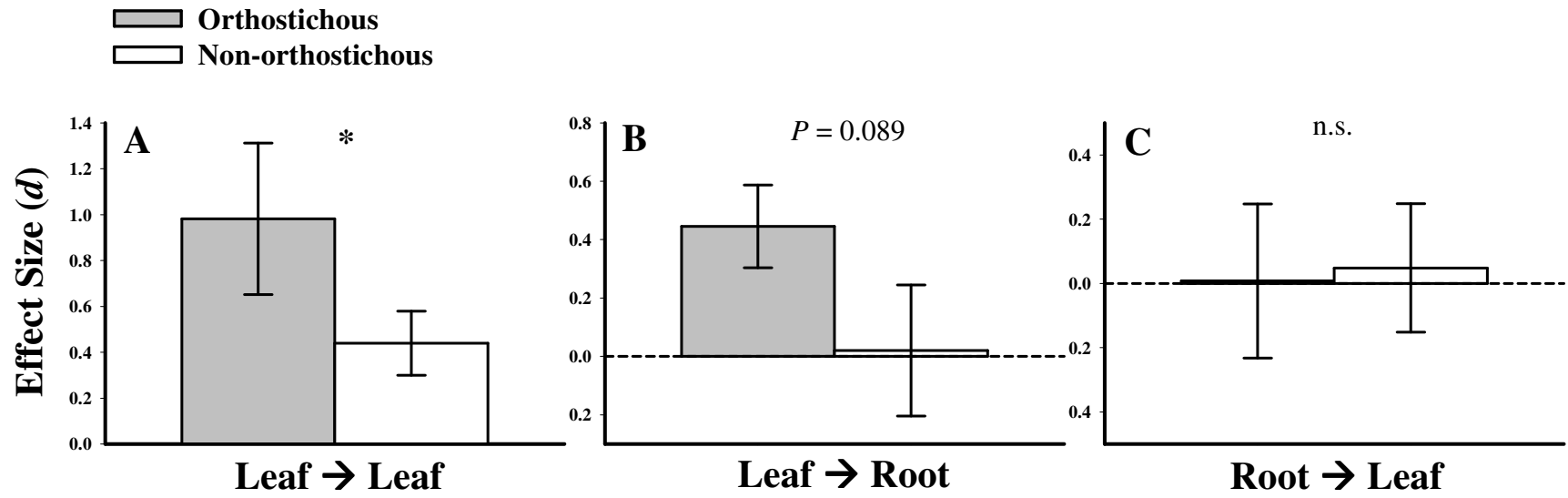


Figure 3.4



CHAPTER 4: The importance of roots in foliar plant defenses and consequences for above-belowground interactions

Abstract

Roots play a critical, but largely unappreciated, role in aboveground anti-herbivore plant defenses and root-leaf connections may therefore result in unexpected coupling between above- and belowground consumers. Using the tobacco (*Nicotiana tabacum*) system I highlight two examples of this phenomenon. First, the chemical nicotine is produced in roots, yet expressed as a foliar resistance trait. I demonstrate that nematode root herbivory interferes with foliar nicotine expression, resulting in positive effects on aboveground phytophagous insects. Notably, nematode-induced facilitation only occurred on nicotine-expressing plants, and not on nicotine-deficient mutants. In the second case, I use pulse-labeling (^{13}C) of plants followed by stable isotope analysis to demonstrate that foliar herbivory elicits a tolerance response whereby aboveground nutritional reserves are allocated to roots, resulting in facilitation of phytoparasitic nematodes. Thus, plants integrate roots in resistance and tolerance mechanisms for leaf defense, and such root-leaf connections inherently link the dynamics of above- and belowground consumers.

Introduction

The fundamental role of roots in terrestrial plants is to acquire growth-limiting water and nutrients (e.g., nitrogen) from the surrounding environment (Öpik and Rolfe 2005), although plants also utilize roots to cope with a more diverse array of biotic and

abiotic challenges (McCully 1999, Bais et al. 2004, Schenk 2006). One such challenge that plants frequently encounter is attack from consumers at the 2nd trophic level. Despite the fact that all plant tissues (i.e., leaves, roots, stems, fruits) are susceptible to herbivory, the vast majority of knowledge on plant-herbivore interactions derives from studies on leaf consumption (e.g., Johnson et al. 2006). Because leaves and roots are spatially separated from one another, often by great distances, the contribution of roots to leaf defense and patterns of aboveground herbivory is not widely recognized. Water stress and nutrient availability have well-documented effects on the preference and performance of leaf-feeding insects (Herms 2002, Huberty and Denno 2004), and consequently roots have been indirectly implicated in aboveground plant-herbivore dynamics. However, roots play a more direct, albeit less apparent, role in foliar plant defenses, and I argue that these leaf-root connections have important ecological consequences in linking above- and belowground consumers.

Because roots are surrounded by soil and thus inaccessible to most foliar herbivores, they provide the ideal storage site for resources used in aboveground defense. We highlight two examples of this phenomenon, one a resistance trait (Fig. 4.1A) and the other a tolerance mechanism (Fig. 4.1B). First, secondary plant compounds that protect leaves from consumers are often synthesized in root tissue, thus providing a direct link between roots and foliar resistance (Karban and Baldwin 1997, van der Putten et al. 2001). For example, plants in the genus *Nicotiana* employ alkaloids (e.g., nicotine) as a constitutive and inducible defense against leaf-chewing herbivores (Baldwin 1991, Steppuhn et al. 2004). Notably, alkaloid biosynthetic sites are located entirely belowground in tobacco roots, despite their well-established use in foliar defense

(Dawson 1941, 1942, Baldwin 1988). Similar integration between roots and leaf resistance can be observed in other plant defense systems such as cotton (*Gossypium* sp.), which synthesizes terpenoid aldehydes (e.g., gossypol) in roots (Smith 1961; Heinstein *et al.* 1962), yet expresses these compounds aboveground where they negatively affect foliar-feeding insects (Meisner *et al.* 1978, Parrott 1990, Agrawal and Karban 2000).

In addition to providing a “safe haven” for the biosynthesis of secondary compounds, roots have recently been implicated in tolerance to foliar herbivores by providing a temporary storage site for primary metabolic products (e.g., photoassimilates) that would otherwise be vulnerable to aboveground consumers (Babst *et al.* 2005, Schwachtje *et al.* 2006). In these cases, the sink strength of roots is elevated when leaves are induced, thereby increasing belowground allocation of plant metabolites. Nutritional resources can then be re-allocated at a later time for aboveground growth and/or reproduction.

The logic underlying the resistance and tolerance mechanisms described above presumes that roots are less susceptible to herbivory than leaves (Karban and Baldwin 1997, van der Putten *et al.* 2001). However, a large and diverse group of cryptic soil-dwelling consumers (e.g., nematodes, arthropods, mammals) subsist largely on plant roots (Brown and Gange 1990, Coleman *et al.* 2004). As a result, when plants simultaneously host above- and belowground herbivores, unexpected interactions may occur between these two groups of consumers. Although recent studies emphasize the role of induced defenses in plant-mediated competition between foliar and root herbivores (van Dam *et al.* 2003, Bezemer *et al.* 2003, 2004, Bezemer and van Dam 2005, Soler *et al.* 2005, van Dam *et al.* 2005, van Dam and Bezemer 2006, Kaplan and

Denno 2007, Soler et al. 2007), I demonstrate that leaf-root connections can generate the opposite effect, with positive interactions linking foliar and root consumers. When secondary chemicals are synthesized belowground, root herbivory can interfere with aboveground expression, leading to positive effects on foliar herbivores (Fig. 4.1A). Similarly, if aboveground herbivores elicit a tolerance response whereby plants allocate valued nutritional resources belowground, this storage effect may benefit root herbivores (Fig. 4.1B). I present experimental evidence for such reciprocal positive interactions between foliar and root herbivores on tobacco (*Nicotiana tabacum*), a system in which roots are known to be involved in anti-herbivore resistance (Dawson 1941, 1942) and tolerance (Schwachtje et al. 2006). Thus, plant defense strategies that integrate discrepant tissues, such as leaves and roots, may be a common feature of how plants cope with consumers and provide novel opportunities for linking above- and belowground biota.

Methods

Foliar insect performance on plants with nematode root herbivory

The impact of nematode root herbivory (*Meloidogyne incognita*) on foliar-feeding insect herbivores (*Trichoplusia ni* and *Manduca sexta*) of tobacco was tested, with the expectation that insects would perform better on plants with experimentally-imposed nematode herbivory via foliar nicotine interference (Fig. 4.1A). The nematode, *M. incognita*, is a gall-forming species that is largely considered the dominant parasite of tobacco roots (Barker and Lucas 1984), whereas *T. ni* and *M. sexta* are generalist and specialist caterpillars, respectively, that defoliate tobacco leaves. The difference in host range of the two caterpillars is important to note because the specialist, *M. sexta*, can

detoxify and excrete tobacco alkaloids (Wink and Theile 2002), and therefore is less sensitive to nicotine in its diet when compared with the generalist *T. ni* (Krischik et al. 1991). Thus, if nematode root herbivory benefits leaf-chewing insects via aboveground nicotine interference, then the effect should be relatively stronger for *T. ni* than for *M. sexta*.

Tobacco plants (var. MD 609) were propagated by seed in a greenhouse and seedlings (9 weeks of growth from seed to seedling stage) were subsequently transplanted singly into 4-gallon pots containing a sterilized growing medium [50% sand, 50% potting mix (SunGro LC1 and professional blend; sphagnum peat moss, bark, perlite, vermiculite, and clay)]. Plants were supplemented with nutrients weekly using a soluble fertilizer (20:10:20 NPK). The roots from half of all seedlings were randomly assigned to root herbivory and inoculated at the time of transplant with ~100,000 *M. incognita* eggs obtained from a laboratory culture. This egg density is well within the range documented for *M. incognita*-infested tobacco fields (Barker and Lucas 1984) and also corresponds with inoculum levels used in prior studies (e.g., Hanounik and Osbourne 1975, 1977, Barker and Weeks 1991). The remaining plants that did not receive experimental addition of nematode eggs served as controls.

Three weeks after nematode-inoculation, caterpillars were added to the leaves of control and root herbivory plants. Hornworms (*M. sexta*) were obtained from a local colony (NC State Univ) and cabbage loopers (*T. ni*) from a biological supply company (Benzon Research, Inc., Carlisle, PA, USA). Second-instar larvae of each species were independently reared on plants, with each plant receiving a single caterpillar. After seven days of growth, caterpillars were removed from plants and weighed. The experiment

involving *T. ni* was repeated at three different times, with 42 replications each of control and root herbivory plants. The experiment with *M. sexta* was repeated at four different times, with 45 replications each of control and root herbivory plants.

In addition to measuring insect performance, the alkaloid content of foliage was determined from samples of newly-expanding leaves. Samples were immediately frozen in liquid nitrogen and extracted and analyzed by HPLC (Keinänen et al. 2001) on a reversed phase C18 column (Gemini C18, 150 x 4.6 mm; Phenomenex). Concentrations of foliar nicotine were quantified using calibration curves prepared from commercially available standards.

Differences in caterpillar weights (mg wet weight) and leaf nicotine content between control and nematode-inoculated plants were assessed using ANOVA, with date as a random effect (Proc mixed; statistical analyses were performed using SAS, Version 9.1; SAS Institute, Inc., 2001). Data were square-root transformed prior to statistical analysis to meet assumptions of normality and homogeneity of variances.

The role of nicotine in nematode-induced effects on foliar insect herbivores

If nicotine is indeed the causal mechanism underlying nematode-induced effects on aboveground caterpillars, then nematode root herbivory should affect caterpillar performance on nicotine-expressing plants, but not on nicotine-deficient plants. I tested this hypothesis using two near-isogenic lines of tobacco that differ in nicotine content. Burley 21 is a wild-type, nicotine-expressing tobacco line, whereas LA Burley 21 has mutations at the two loci controlling nicotine expression and thus is nicotine-deficient (Hibi et al. 1994). Seeds for the two lines were obtained through the USDA National

Plant Germplasm System (NPGS) and cultivated in a greenhouse as described above. Plants that differed in nicotine content were fully-crossed in a 2 x 2 factorial design with nematode root herbivory (as described above), resulting in four different treatment combinations (n=38 replications split over two dates).

Second-instar larvae of the generalist caterpillar *Spodoptera exigua* were added singly to each plant three weeks after nematode-inoculations, and caterpillars were removed from plants and weighed after seven days of growth. In addition to measuring caterpillar performance, I also assessed the quantity of leaf tissue damaged by caterpillars using an acetate grid to estimate defoliation.

To ensure that nicotine did not affect nematode root herbivory, and thus confound the two treatment effects, nematode performance on nicotine-expressing and nicotine-deficient tobacco lines was also evaluated. After final caterpillar performance and defoliation measurements, plant roots were removed and nematode eggs harvested using a modified version of the Hussey and Barker (1973) extraction procedure. Performance was assessed by counting nematode eggs and fecundity was compared between plants that did and did not express nicotine. Additionally, leaf tissue samples were harvested and analyzed for secondary chemicals (e.g., nicotine, non-nicotine alkaloids, chlorogenic acid, caffeoyl putrescine) using the HPLC procedure described above (see Keinänen et al. 2001) to confirm that the two tobacco lines differed in nicotine content, but not in other compounds that potentially affect plant quality for herbivores. Last, because nicotine is a nitrogen-rich compound, I also measured % nitrogen in the leaves of both plant types using a CHN elemental analyzer.

ANOVA was used to compare caterpillar weights (mg wet weight) and defoliation (cm² of leaf tissue removed) on control and root herbivory plants, with date as a random effect in the model (Proc mixed). Separate analyses were conducted for nicotine-expressing and nicotine-deficient tobacco lines. I also compared nematode fecundity, secondary chemistry, and % nitrogen between plants that did and did not express nicotine using ANOVA (Proc mixed). Caterpillar larval weights were log transformed prior to statistical analysis.

Nematode performance on plants with foliar insect herbivory

The reciprocal effect of aboveground insect herbivory on belowground nematode performance was assessed with the expectation that nematodes would perform better when co-occurring with foliar-feeding insects (Fig. 4.1B). Tobacco plants were cultivated and inoculated with nematode eggs (~10,000 eggs/plant) as described above. These plants were randomly assigned to one of four treatments: (1) undamaged control, (2) *M. sexta* herbivory, (3) *T. ni* herbivory, and (4) mechanical damage (n=12 replications per treatment). Plants in the *M. sexta* and *T. ni* herbivory treatments were defoliated by caterpillars at three times during the course of the experiment (two, five, and eight weeks after nematode inoculations). For each defoliation bout, a single third-instar *M. sexta* larva or five fourth-instar *T. ni* larvae were added to each plant and removed after four days. Caterpillar leaf damage on each plant was estimated using an acetate grid. Plants in the mechanical-damage treatment were defoliated using a hole-punch and the quantity and timing of tissue damage coincided with the caterpillar herbivory treatments. Ten weeks after nematodes were added to plants, roots were collected and nematode

performance was measured by harvesting and counting eggs as described above.

ANOVA followed by Tukey-Kramer's test for means separation (Proc glm) was used to assess the impact of defoliation (cm^2 of leaf tissue removed) on nematode performance.

Pearson's product-moment correlation was used to determine the relationship between overall levels of caterpillar feeding damage and nematode fecundity (Proc corr).

Leaf-root carbon allocation on plants with above- and belowground herbivory

Foliar herbivory is predicted to affect root herbivores via changes in above- and belowground resource allocation, with an increase in root sink strength on plants with aboveground herbivory (Fig. 4.1B). To test this hypothesis I manipulated foliar (*M. sexta*) and root (*M. incognita*) herbivores in a 2 x 2 factorial design (n=15 replications per treatment combination), and used stable isotope analysis to quantify the effects of herbivory on within-plant carbon translocation. Nematodes were inoculated on greenhouse-grown plants as described above and three weeks later a single second-instar *M. sexta* larva was added to those plants assigned to the foliar herbivory treatment. After five days of leaf damage, all plants were enriched using the carbon isotope ^{13}C . Because ~99% of carbon in terrestrial ecosystems occurs in the form ^{12}C , plants can be pulse-labeled using the heavier (^{13}C) isotope as a physiological tracer (Dawson et al. 2002). The first fully-expanded leaf on each plant was enclosed in an air-tight chamber and exposed to $^{13}\text{CO}_2$ by reacting 38 mg of sodium bicarbonate, $\text{NaH}^{13}\text{CO}_3$ (99 atom% ^{13}C ; Sigma-Aldrich, Milwaukee, WI, USA), with 500 μl lactic acid for 90 minutes. Leaf chambers were removed and 48 hrs later all emerging leaves (i.e., those occurring above the source-sink transition leaf) and roots were harvested. Plant tissues were dried, ground, and

analyzed for carbon isotopic signatures using an isotope ratio mass spectrometer (Cornell University Stable Isotope Laboratory, Ithaca, NY, USA). Two blocks of the experiment (8 total plants) were not enriched to assess ambient levels of ^{12}C and ^{13}C .

Average ambient levels of ^{13}C in non-enriched plants were subtracted from ^{13}C levels in labeled plants such that analyzed and presented ^{13}C values represent actual quantities of carbon that were assimilated and translocated via the labeling process. The impact of foliar and root herbivory on leaf-root ^{13}C allocation was assessed using two-way ANOVA, with insect and nematode herbivory as main effects (Proc mixed). I independently analyzed the impact of herbivory on carbon allocation to leaves, roots, and the root:leaf ratio. Spatial groupings of plants (i.e., blocks of each replicate) were considered as a random effect in the model.

Results

Foliar insect performance on plants with nematode root herbivory

Belowground nematode-feeding increased larval weight gain in the generalist caterpillar *T. ni* by 29% ($F_{1,74} = 6.19$, $P = 0.0151$; Fig. 4.2A). However, root herbivory did not affect the performance of the specialist caterpillar *M. sexta* ($F_{1,80} = 2.19$, $P = 0.1425$; Fig. 4.2B), although there was a trend for greater weight gain on root herbivory plants. The concentration of leaf nicotine was >2x higher on control than on root herbivory plants, regardless of whether *T. ni* ($F_{1,26} = 19.12$, $P = 0.0002$; Fig. 4.2C) or *M. sexta* ($F_{1,25} = 38.16$, $P < 0.0001$; Fig. 4.2D) was the aboveground herbivore.

The role of nicotine in nematode-induced effects on foliar insect herbivores

The foliar nicotine content of nicotine-expressing tobacco lines was >5x higher than nicotine-deficient plants ($F_{1,18} = 52.06$, $P < 0.0001$). However, the two tobacco lines did not differ in nematode performance ($F_{1,16} = 0.02$, $P = 0.8998$), non-nicotine secondary chemistry (non-nicotine alkaloids: $F_{1,18} = 2.80$, $P = 0.1118$, chlorogenic acid: $F_{1,18} = 2.08$, $P = 0.1662$, caffeoyl putrescine: $F_{1,18} = 0.04$, $P = 0.8385$), or % foliar nitrogen ($F_{1,38} = 0.82$, $P = 0.3699$).

Nematode root herbivory increased the weight gain of the caterpillar *S. exigua* on nicotine-expressing plants ($F_{1,75} = 6.10$, $P = 0.0158$; Fig. 4.3A), but had no impact on caterpillar performance on nicotine-deficient plants ($F_{1,71} = 0.00$, $P = 0.9902$; Fig. 4.3A). Similarly, caterpillars removed more leaf tissue on nicotine-producing plants with nematode root herbivory ($F_{1,21} = 7.43$, $P = 0.0126$; Fig. 4.3B), but nematode presence had no impact on caterpillar defoliation on nicotine-deficient plants ($F_{1,19} = 0.13$, $P = 0.7244$; Fig. 4.3B).

Nematode performance on plants with foliar insect herbivory

Aboveground insect herbivory elevated the fecundity of nematodes feeding belowground by 44%, an effect that was equally strong for the generalist and specialist caterpillar species ($F_{3,38} = 3.15$, $P = 0.0361$; Fig. 4.4A). Notably, mechanical damage using a hole-punch did not elicit the same positive response in nematodes as caterpillar defoliation. Overall, the level of leaf damage by aboveground herbivores was positively correlated with nematode egg production ($r = 0.5028$, $P = 0.0171$; Fig. 4.4B).

Leaf-root carbon allocation on plants with above- and belowground herbivory

Neither caterpillars ($F_{1,43} = 1.70$, $P = 0.1991$) nor nematodes ($F_{1,43} = 2.59$, $P = 0.1149$) affected the allocation of ^{13}C to developing tobacco leaves (Fig. 4.5A). However, aboveground caterpillar herbivory increased ^{13}C allocation to roots ($F_{1,42} = 4.17$, $P = 0.0474$), whereas nematode root herbivory had no impact ($F_{1,42} = 2.60$, $P = 0.1142$; Fig. 4.5B). Similarly, the ^{13}C root:leaf ratio was higher (i.e., greater belowground allocation) on plants with foliar insect herbivory ($F_{1,42} = 4.97$, $P = 0.0312$), whereas nematodes did not affect the root:shoot ratio ($F_{1,42} = 0.01$, $P = 0.9306$; Fig. 4.5C). Interactions between foliar and root herbivory were non-significant for all response variables.

Discussion

Because plant roots are concealed belowground and spatially separated from leaves, their contribution to aboveground anti-herbivore plant defense strategies is not generally acknowledged. However, I experimentally demonstrate that roots are intimately involved with leaf defense against phytophagous insects and that such leaf-root connections mediate indirect interactions between above- and belowground consumers.

In the case of foliar resistance, nicotine provides one of the best examples of leaf-root integration (Fig. 4.1A). Not only is nicotine root biosynthesis well-established (Dawson 1941, 1942, Baldwin 1988), but the ecological relevance of nicotine as an aboveground defense is also well-documented (Baldwin 1991, Steppuhn et al. 2004). Nematode root herbivory is known to suppress the constitutive and inducible foliar expression of nicotine in tobacco (Hanounik and Osbourne 1975, 1977, Barker and Weeks 1991, Kaplan et al. in press), and therefore I predicted that root-feeding herbivores would induce susceptibility to leaf-feeding insects by interfering with aboveground

nicotine dynamics. Indeed, caterpillars performed better when co-occurring on plants with nematode root herbivores (Figs. 4.2A and B). Several lines of evidence implicate nicotine as the causal mechanism underlying this effect. First, nematodes had a relatively greater impact on the performance of the nicotine-sensitive generalist caterpillar *T. ni* than on the nicotine-tolerant specialist *M. sexta*. Second, leaf nicotine content was >50% lower on plants incurring root herbivory (Figs. 4.2C and D). Last, nematodes only affected caterpillar performance (Fig. 4.3A) and defoliation (Fig. 4.3B) on nicotine-expressing tobacco lines, whereas no interaction occurred on nicotine-deficient mutant plants.

Thus, despite the emphasis on induced defenses linking leaf and root herbivores (van Dam et al. 2003, Bezemer et al. 2003, 2004, Bezemer and van Dam 2005, Soler et al. 2005, van Dam et al. 2005, van Dam and Bezemer 2006, Kaplan and Denno 2007, Soler et al. 2007), the opposite effect can occur in systems where aboveground allelochemicals are produced belowground. In a recent study, root herbivory was found to interfere with the foliar expression of root-derived secondary chemicals, but elevate the foliar expression of compounds that are synthesized in leaves (Kaplan et al. in press). As a result, the location of biosynthetic sites for ecologically-important phytochemicals may ultimately dictate whether root herbivory leads to net positive or negative effects on foliar consumers. Additionally, the amount of damage inflicted on roots will likely affect the outcome of the interaction, with stronger aboveground interference occurring on plants with more intense root herbivory (Preisser et al. 2007; Kaplan et al. in press). Last, I suspect that herbivore feeding guild may be a contributing factor to interaction strength. The focal root herbivore in this study is a gall-forming nematode whose feeding style and

effects on root morphology and physiology are entirely different than those induced by chewing insects, which to date are vastly over-represented in the literature on plant-mediated interactions (Bezemer and van Dam 2005, van Dam and Bezemer 2006, Kaplan and Denno 2007).

The second means by which roots are integrated with aboveground defense is through plant tolerance responses whereby foliar nutritional resources are preferentially allocated to roots (Fig. 4.1B; Babst et al. 2005, Schwachtje *et al.* 2006). Unlike the above-described resistance trait which resulted in unidirectional plant-mediated effects of nematodes on insects, herbivore-induced tolerance responses mediate the reciprocal effect of insects back onto nematodes. In this case I found that nematode root herbivores benefited from sharing plants with leaf-feeding insects (Fig. 4.4), and this positive response was associated with an increase in root sink strength for photoassimilates elicited by caterpillar feeding (Figs. 4.5B and C). Because sink strength is known to affect the success of herbivores, especially vascular tissue feeders, aboveground caterpillar defoliation likely facilitated root-galling nematodes by redirecting assimilate flow within the plant (Rehill and Schultz 2003, Denno and Kaplan 2007). Moreover, the positive effect of defoliation on nematode fecundity was only elicited in response to actual herbivory and not in response to simple mechanical damage (i.e., hole-punch). This finding suggests that some feature unique to caterpillar feeding (e.g., saliva) caused this effect to occur, as has been shown for other systems (e.g., Halitschke et al. 2003). Notably, a recent study documenting similar tolerance responses found an increase in root sink strength when plants were treated with caterpillar (*M. sexta*) regurgitant and not in response to simple tissue damage (Schwachtje et al. 2006).

Conclusions

Although the defensive phenotype of plant leaves against consumers is influenced by several ecological and evolutionary factors (e.g., resource availability, genotype, etc.; Herms and Mattson 1992), the within-plant contribution of roots is not generally considered. However, I offer two distinct examples of how roots contribute to aboveground plant defenses. Importantly, the mechanisms by which these two defenses function are extremely different, one a neurotoxic chemical that poisons consumers, and the other a means for re-allocating and thus protecting valued nutritional reserves. Yet in both cases roots are directly involved in the process. Also, each example of leaf-root integration results in ecological consequences when plants simultaneously host above- and belowground consumers. Because root-feeding nematodes, and other belowground phytoparasites, are ubiquitous components of the soil environment (Brown and Gange 1990, Coleman et al. 2004), herbivore-induced facilitation may indeed be an important feature linking the dynamics of above- and belowground communities.

Figure Legends

Figure 4.1. A mechanistic model depicting circumstances in which roots function in foliar plant defenses (A: resistance, B: tolerance), and thus promote indirect interactions between above- and belowground herbivores. (A) Because many ecologically-important secondary plant chemicals are synthesized in roots and secondarily transported to leaves, root herbivory may interfere with the aboveground expression of allelochemistry and improve the performance of foliar herbivores. (B) Plants may tolerate foliar herbivory by temporarily storing valuable plant reserves in roots where they can not be accessed by aboveground consumers, and in so doing improve the quality of root tissue for belowground consumers. Solid lines indicate direct effects, and dashed lines denote indirect effects.

Figure 4.2. Impact of nematode root herbivory (+ or -) on the (A) performance (larval mass) of the generalist caterpillar *T. ni*, (B) performance (larval mass) of the specialist caterpillar *M. sexta*, (C) leaf nicotine content of *T. ni*-damaged plants, and (D) leaf nicotine content of *M. sexta*-damaged plants (means \pm SE). Asterisks denote significant differences between means ($P < 0.05$); n.s. = non-significant.

Figure 4.3. Interactive effects of nematode root herbivory and nicotine on (A) caterpillar performance (larval weight), and (B) caterpillar leaf damage (means \pm SE). Nicotine (+) = nicotine-expressing tobacco line and nicotine (-) = nicotine-deficient tobacco line; n.s. = non-significant.

Figure 4.4. Consequences of foliar herbivory for the performance (fecundity) of root-feeding nematodes. (A) Nematode egg production on plants with caterpillar herbivory (the specialist *M. sexta*, and the generalist *T. ni*) and mechanical damage (hole punch). Means (\pm SE) with different letters are significantly different ($P < 0.05$). (B) Relationship between quantity of leaf tissue damaged by caterpillars and nematode fecundity.

Figure 4.5. Effects of foliar and root herbivory on above- and belowground carbon allocation. Plants were pulse-labeled with ^{13}C and stable isotope analysis was used to measure the impact of leaf and root herbivory on whole-plant carbon partitioning, including (A) leaves, (B) roots, and (C) root:leaf ratio (means \pm SE). Significance of the main effects of foliar insect and root nematode herbivory are indicated for each response variable; n.s. = non-significant.

Figure 4.1

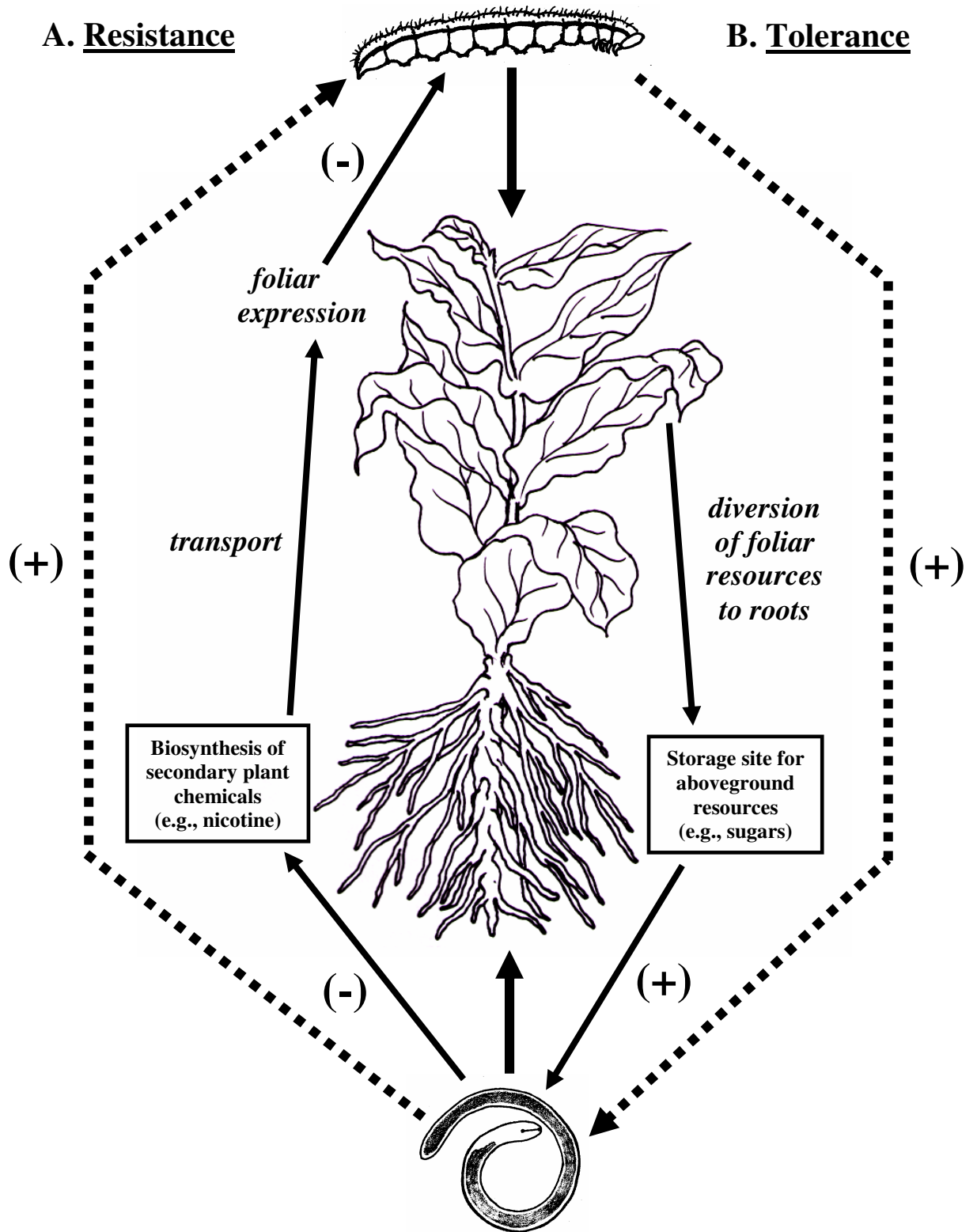


Figure 4.2

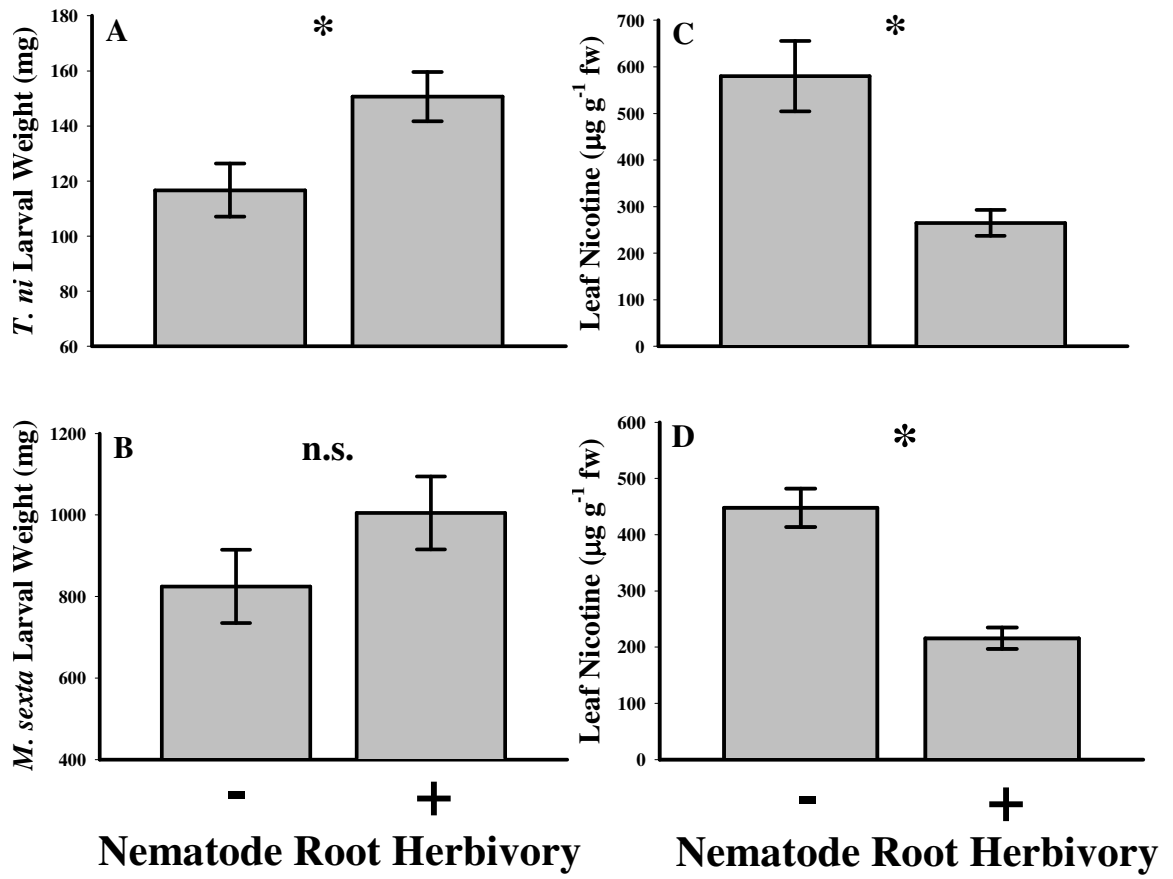


Figure 4.3

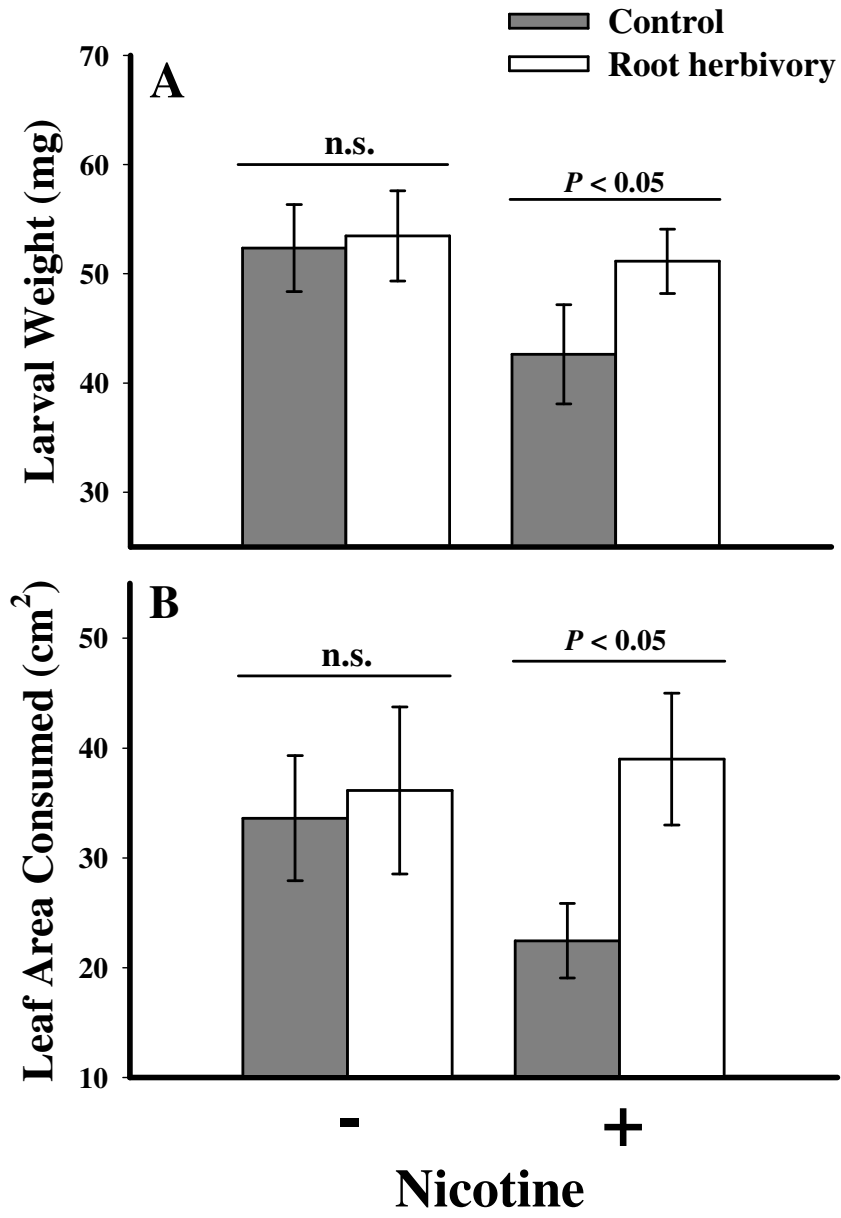


Figure 4.4

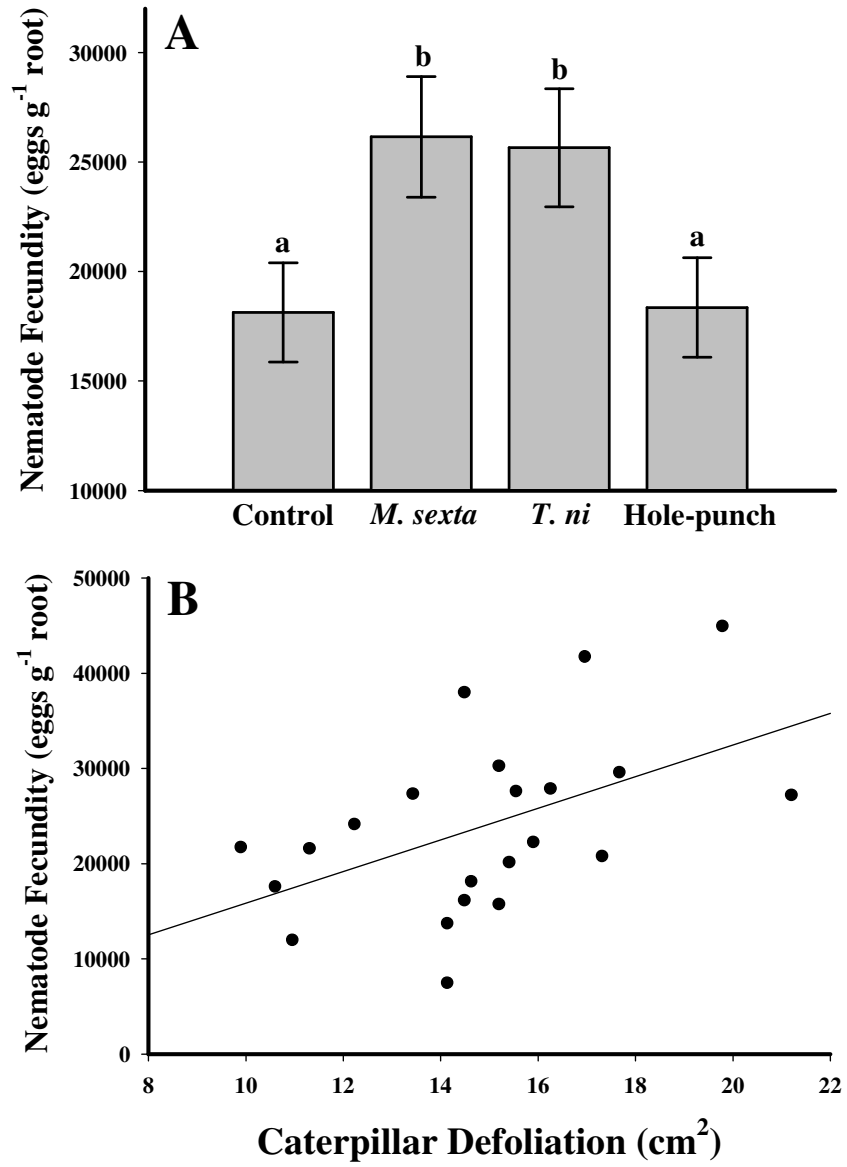
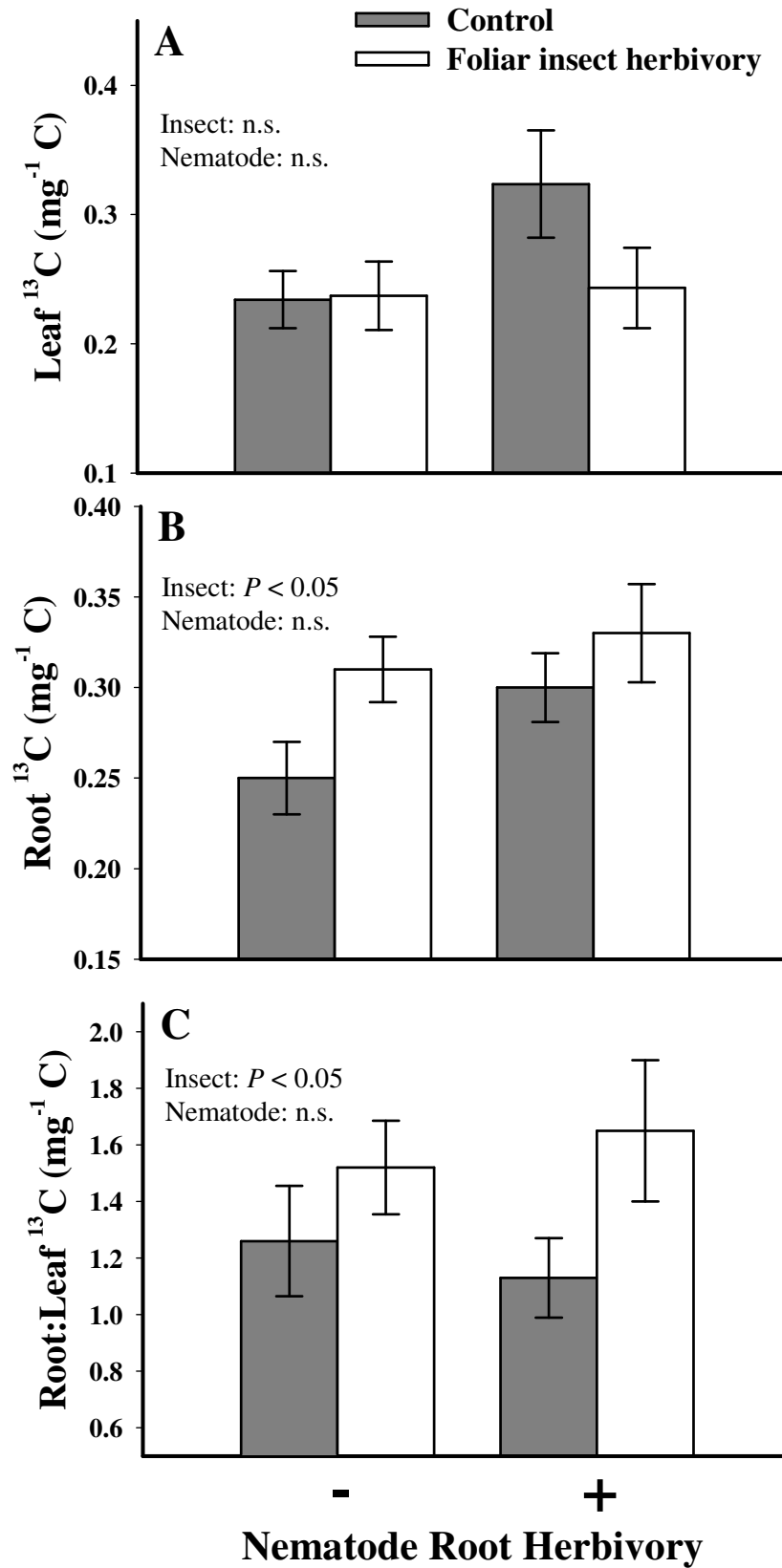


Figure 4.5



Chapter 5: Plant-mediated interactions link foliar-feeding insect and root-feeding nematode communities

Abstract

Because herbivory often elicits systemic changes in plant traits (e.g., secondary chemistry), indirect herbivore interactions via induced plant responses may be a pervasive feature structuring herbivore communities. Although the ecological importance of this phenomenon has recently been emphasized and described for herbivorous insects, it is unknown if and how induced responses contribute to the organization of other major phytoparasitic taxa. I used a combination of survey and experimental field studies to investigate the role of plants in linking foliar-feeding insects and root-feeding nematodes on tobacco (*Nicotiana tabacum*). Populations of plant-parasitic nematodes were surveyed in the rhizosphere of plants that were naturally defoliated by leaf-chewing insects and on plants where aphid outbreaks occurred. Additionally, I manipulated above- and belowground insect and nematode herbivory, respectively, and measured the response in the opposing community. Plant-mediated interactions between insects and nematodes could be differentiated by insect feeding guild, with positive insect-plant-nematode interactions predominating with leaf-chewing insects and negative interactions occurring with sap-feeding insects. Phytoparasitic nematodes were more abundant in the rhizosphere of insect-defoliated plants, whereas populations were less abundant beneath plants infested with aphids. These results were largely consistent across survey and manipulative field experiments. When caterpillar defoliation was experimentally imposed on field-grown plants, the belowground abundance of the tobacco-feeding ectoparasite

Helicotylenchus increased, a response that was detected across different years and sites. Similarly, when root herbivory by the nematode, *Meloidogyne incognita*, was enhanced on field plants, the abundance of certain chewing insect taxa (e.g., *Epitrix* flea beetles) increased, although the density of others such as tobacco hornworms (*Manduca sexta*) was unaffected. Consequently, overall levels of insect defoliation were no different on root herbivory treatments. Early-season aphid populations were lower on nematode-inoculated plants, an effect, however, that did not persist later in the season. Overall, results from these field studies suggest that systemic plant responses to herbivory indirectly link the dynamics of divergent consumer taxa in spatially-distinct ecosystems. This lends support to the growing perception that plants play a critical role in propagating indirect effects among a diverse assemblage of consumers.

Introduction

Real-world ecological communities rarely (if ever) function as discrete units, independent from neighboring habitats. Perhaps the most pervasive opportunity for two “separate” communities to influence one another occurs at the soil-air interface. Virtually all terrestrial plants possess above- and belowground tissues that respond to their respective environments. Accordingly, factors that influence how plants uptake water and nutrients in their roots should have a strong impact on organisms that are associated with shoots (e.g., Masters et al. 1993). Similarly, heterotrophs that interfere with aboveground photosynthetic processes can indirectly alter the structure of belowground food webs (Bardgett et al. 1998). As a result, plants serve as a conduit indirectly linking the dynamics of two spatially-separated communities, and one may thus expect reciprocal

interactions between above- and belowground biota mediated by plant responses to their foliar and root environments (van der Putten et al. 2001, Wardle 2002, Wardle et al. 2004a).

Although numerous pathways can mediate above/belowground linkages, inducible plant responses provide a likely mechanism connecting foliar and root herbivore communities (van Dam et al. 2003, Bezemer and van Dam 2005, van Dam and Bezemer 2006, Kaplan et al. in press). Oftentimes induced responses are transmitted via plant hormones (e.g., jasmonic acid) that elicit systemic changes in anti-herbivore resistance traits (Omer et al. 2000, van Dam et al. 2001, 2004). Thus, herbivory in leaves or roots can induce whole-plant changes in allelochemistry (Bezemer et al. 2004, van Dam and Raaijmakers 2006, Kaplan et al. in press), resulting in indirect effects on co-occurring species (Bezemer et al. 2003, Soler et al. 2005, van Dam et al. 2005, Soler et al. 2007, Kaplan et al. in review). In addition, many induced secondary plant chemicals (e.g., phenolics) have broad-spectrum efficacy and deter a wide range of consumers (Karban and Baldwin 1997). Therefore, dissimilar organisms (e.g., insects, nematodes, fungi) with diverse feeding styles may nonetheless affect one another (Stout et al. 2006, Kaplan and Denno 2007). This issue is particularly relevant to considerations of above/belowground interactions as substantial taxonomic differences often exist in the composition of foliar- and root-associated communities (Scott et al. 1979, Stanton 1988). Last, induced responses to herbivory in leaves and roots have been linked with whole-plant changes in the translocation of primary plant metabolites that alter the nutritional quality of above- and belowground tissues (Babst et al. 2005, Schwachtje et al. 2006, Newingham et al. 2007).

Plant-parasitic nematodes and phytophagous insects comprise a large proportion of the biodiversity and abundance of multi-cellular animal life on earth (Sohlenius 1980, Strong et al. 1984). Nematodes dominate the belowground herbivore community (attaining densities $>1,000,000$ individuals/m²) on many plant species where they impair the translocation of water/nutrients and thus limit primary production (Smolik 1977, Stanton 1988, Ingham and Detling 1990). In North American grasslands, for example, nematodes have been identified as the key consumer (either above- or belowground) controlling plant productivity (Scott et al. 1979, Stanton et al. 1981, Stanton 1988). Moreover, the role of nematodes in reducing agricultural yield has been well-documented, with annual losses estimated at $> \$100$ billion worldwide (Sasser and Freckman 1987). Phytophagous insects are the aboveground counterparts to nematodes where they are well-recognized for their detrimental effects on plants in natural and managed habitats (Bardner and Fletcher 1974, Marquis 1992). Both nematodes and insects are present (and typically abundant) in virtually all terrestrial ecosystems, yet potential interactions between these two dominant groups of plant parasites are poorly described. Mounting evidence, however, suggests that insect-plant-nematode interactions may indeed occur (Russin et al. 1989, Alston et al. 1991, Russin et al. 1993, Alston et al. 1993, Carter-Wientjes et al. 2004, Wardle et al. 2004b, van Dam et al. 2005, De Deyn et al. 2007, Wurst and van der Putten 2007, Kaplan et al. in review). Because most of the above studies were conducted in controlled environments, the ecological relevance of plant-mediated interactions linking insect and nematode populations in complex communities remains unclear. In addition, previously published accounts have focused exclusively on unidirectional interactions (i.e., the impact of nematodes on insects, or

vice versa); notably, none has investigated reciprocal dynamics. Towards this end, I initiated a series of survey and manipulative field studies aimed at quantifying the reciprocal nature of plant-mediated insect-nematode interactions in the field. Testing whether or not such above-belowground linkages can be discerned under realistic field conditions despite wide-spread heterogeneity in biotic and abiotic variables is a critical step in this emerging ecological field.

Methods

Study system

The phytophagous insect and nematode community associated with the agricultural crop plant tobacco (*Nicotiana tabacum*) was used as my study system. Tobacco is cultivated throughout the mid-Atlantic United States and hosts a diverse assemblage of foliar- and root-feeding herbivores. The aboveground herbivorous insects of tobacco can be partitioned into two primary feeding guilds, chewers and sap-feeders. Among the chewers, the Solanaceae-specialist *Manduca sexta* is the most abundant and damaging insect, but *Epitrix* sp. flea beetles often reach high densities as well. Moreover, a variety of sub-dominant generalist caterpillars (i.e., *Heliothis virescens*, *Spodoptera exigua*) are also present. Of the sap-feeders, the aphid *Myzus persicae* is the dominant species, outnumbering all other sap-feeding insects by several orders of magnitude. The belowground phytophagous nematodes of tobacco are relatively diverse, consisting of several genera and feeding guilds. The sedentary endoparasite, *Meloidogyne incognita*, is a polyphagous and gall-inducing species that is considered the primary nematode pest on

tobacco (Barker and Lucas 1984). However, several genera of tobacco-feeding ectoparasites were consistently present in local agricultural fields.

Patterns of insect and nematode co-occurrence – field survey

To test whether above- and belowground herbivores co-occur on plants more or less frequently than expected, I surveyed tobacco fields that were naturally colonized by insects and nematodes to assess patterns of foliar and root herbivory. In 2006 I surveyed insects and nematodes in two adjacent tobacco fields (each field ~500 plants; var. MD609) at the Central Maryland Research and Education Center (Upper Marlboro, MD). Both fields were cultivated with standard agronomic inputs of nitrogen, but were not irrigated or treated with insecticides.

In field one, I tested for an association between aboveground insect defoliation and belowground nematode herbivory. Tobacco plants were sampled for insect herbivory in late-June (~1 month after seedlings were transplanted) and divided into one of two groups: (a) undamaged (n=36), or (b) insect-defoliated (n=44). Undamaged plants had virtually no evidence of herbivory (<1% leaf area removed), whereas insect-defoliated plants received substantial damage from leaf-chewing insects (20-60% leaf area removed). Plants were distributed such that no two observations within each group were <3 m from one another, and oftentimes the between-plant distances were far greater. Furthermore, plants in the two groupings were interspersed and thus undamaged plants were frequently neighboring defoliated ones. All plants were uprooted and the rhizosphere soil adhering to the roots was collected for nematode extraction.

In field two, I tested for an association between aboveground sap-feeding insects (aphids) and belowground nematode herbivory. Tobacco aphids (*M. persicae*) are late-season feeders and attain peak densities between late-July and early-August. Aphid outbreaks, however, are patchily distributed such that outbreak plants (>10,000 aphids per plant) are often adjacent to plants with relatively few aphids (<1,000 individuals). Therefore, I visually surveyed and identified plants with aphid outbreaks and matched them with neighboring plants possessing few aphids. As a result, plant groupings were blocked spatially within the field (n = 20 blocks). Soil samples were collected from the rhizosphere of plants and used to estimate nematode abundance.

Nematodes were extracted from soil (each sample 250 cm³) using a modified version of the Baermann funnel technique (Christie and Perry 1951). Briefly, soil samples were placed in water and subsequently poured onto nested sieves (850- μ m-pore sieve on top of a 45- μ m-pore sieve). The resulting nematodes were placed on Baermann funnels and collected after 48 hrs. All plant-parasitic nematodes present in the samples were identified to genus and counted.

Nematode counts were log transformed and ANOVA was used to compare the abundance of each genus of plant-parasitic nematode beneath (1) insect-defoliated vs. undamaged plants, and (2) aphid outbreak vs. non-outbreak (control) plants (Proc Mixed; all statistical analyses were performed using SAS, Version 9.1; SAS Institute, Inc., 2001). Only nematode genera present in >50% of samples were analyzed for associations with foliar insect herbivory, whereas all genera were pooled to create the variable 'Total phytoparasitic nematode' density. For the aphid assessment, spatial groupings of plants (i.e., blocks) were considered as a random effect.

Impact of foliar insect herbivory on belowground nematode population growth

I conducted a field cage experiment replicated over two years (2006 and 2007) to test the effects of foliar insect herbivory on the population growth of plant-parasitic nematodes. In 2006 I used a field that was previously cultivated in corn for at least five consecutive summers, and in 2007 I used a different field that was cultivated in tobacco during the previous summer (2006) and asparagus in years prior to that. The two fields were located at the same general site and separated by approximately one km. Both fields had a sandy loam soil that was tilled prior to use, neither field was irrigated, and both fields were fertilized at a rate of 100 lbs nitrogen ha⁻¹.

In late-May I erected fine-mesh screen field cages (3.7 x 3.7 x 2.1 m, length x width x height) supported by polyvinyl chloride (pvc) frames, with 3 m separating each cage from neighboring cages. In 2006, I constructed 14 cages, whereas 15 cages were used in 2007. Aside from this minor difference in replication, the experimental protocol was virtually identical across the two years. Cages were large enough to enclose four tobacco plants and prevented insects from naturally colonizing experimental plants. Tobacco seedlings were grown from seed in a greenhouse and transplanted into field cages in early-June.

I manipulated the presence of aboveground chewing and sap-feeding insects in a 2 x 2 factorial design, resulting in one of four treatments that was randomly assigned to each seedling per cage: (1) control (no herbivory), (2) caterpillar, (3) aphid, (4) caterpillar and aphid. The two dominant chewing and sap-feeding insects of tobacco, *M. sexta* and *M. persicae*, respectively, were used for our insect manipulations. Caterpillars were

obtained from a locally available colony (NC State University), and aphids were collected from nearby tobacco fields. Insects were added to plants approximately two weeks after seedlings were transplanted into field cages (mid-June). A single 1st instar *M. sexta* larva was added to “caterpillar-defoliated plants”, and 25 aphids were placed on “aphid-infested plants”. Cages were visually surveyed several times per week for the duration of the experiment to ensure that insects remained on their assigned plants. The insect manipulations were largely successful in achieving the desired herbivory treatments and ones that were not (i.e., plants in which caterpillars died and thus did not damage leaves) were excluded from the analysis. On average, caterpillars defoliated 2-3 leaves per plant (20-35% of the total leaf area), whereas aphids attained peak densities of several thousand per plant.

Nematode populations were surveyed in field cages twice during the experiment, once when seedlings were transplanted in early-June and again when the experiment was terminated in late-August. For the early-season sample, 10 soil cores (each core 2.5 x 15 cm, width x depth) were collected from each cage and combined to form a single soil sample that was used to estimate the initial nematode population density per cage. For the late-season sample, tobacco plants were pulled from the ground and the rhizosphere soil adhering to the roots of each plant was collected separately. These samples were used as the final (or post-treatment) assessment of nematode population density associated with each treatment plant.

Nematode population growth was quantified as final density (N_F) divided by starting density (N_0). Because this population growth estimate was not normally distributed, I log transformed the variable. The impact of foliar insect herbivory on

nematode population growth was tested using a two-way mixed model ANOVA (Proc Mixed). Caterpillar and aphid herbivory were considered as fixed effects, whereas year and cage were designated as random effects. Separate ANOVAs were conducted for each taxon of phytoparasitic nematode.

Effects of nematode root herbivory on aboveground insect populations

In 2007 I manipulated root herbivory on field plants to test the reciprocal impact of nematodes on aboveground insect herbivores. A single field of tobacco was cultivated with 150 plants, half receiving supplemental root herbivory (n=75) and the other half serving as controls (n=75). Plants in these two groups were arranged in a completely randomized design and thus root herbivory plants were interspersed with control plants. In previous summers I sampled this field extensively and population densities of plant-parasitic nematodes were low. Thus, I assumed that control plants incurred minor levels of root herbivory.

The gall-forming nematode *M. incognita* was used as my focal root herbivore because it is the dominant nematode parasite of tobacco. Cultures of *M. incognita* originated from local agricultural fields and were reared on greenhouse-grown tobacco prior to use in experiments. Nematode eggs were harvested from the roots of heavily infested plants using a modified version of the Hussey and Barker (1973) procedure, and each seedling assigned to the root herbivory treatment received 100,000 *M. incognita* eggs at transplant. This density is well within the range documented for *M. incognita*-infested tobacco fields (Barker and Lucas 1984) and also corresponds with inoculum levels used in prior studies on *M. incognita*-tobacco interactions (Hanounik and Osbourne

1975, 1977, Barker and Weeks 1991, Vovlas et al., 2004). Because direct nematode inoculations onto field grown plants proved difficult in previous attempts, I first inoculated and grew tobacco seedlings in pots at a greenhouse. After two weeks of growth (mid-June), control and nematode-inoculated seedlings were removed from pots and transplanted into the field. When the experiment was terminated in late-August all plants were removed from the soil and the roots of each plant were inspected for galls to ensure that nematode inoculations were successful and that galls did not occur on control roots.

Plants were surveyed for evidence of aboveground insect activity twice during the summer, once in early-July and again in early-August. On the first sampling date, all tobacco leaves on each plant were visually searched and the identity and abundance of herbivorous insects (eggs, nymphs, larvae and adults) were recorded. On the second sampling date, I assessed foliar damage inflicted on control and experimental plants by leaf-chewing insects, including (a) the proportion of leaf area defoliated, and (b) the number of leaves with >50% leaf area damaged. I also estimated aphid abundance on the second sampling date because aphid densities peak later in the summer than most other tobacco-feeding insects (e.g., hornworms).

The densities of leaf-chewing insects were relatively low. Thus, counts of chewing insects did not follow a normal distribution and consequently I analyzed the effect of nematode root herbivory on chewing insects using Poisson logistic regression (Proc Genmod). Odds ratios were calculated to estimate the likelihood of herbivore occurrence on root herbivory plants relative to control plants (e.g., Van Zandt and Agrawal 2004, Viswanathan et al. 2005). A value of one indicates that insects are equally

abundant on treatment and control plants, whereas odds ratios greater and less than one indicate that insects are more and less abundant, respectively, on plants with experimentally elevated root herbivory. The only chewing insects that occurred frequently enough to statistically analyze were tobacco hornworms, *M. sexta*, and flea beetles in the genus *Epitrix*. Aphid counts were log transformed and compared between treatment and control plants using a t-test (Proc Glm). Separate analyses were conducted for early-season aphid counts when populations were first developing (July) and late-season counts when populations were approaching peak densities (August). Plant damage assessments (% defoliation, and number of damaged leaves) were also analyzed using t-tests (Proc Glm). The proportion of leaf area defoliated was arcsine square-root transformed prior to statistical analysis.

In addition to surveying field plants for colonization and damage by naturally-occurring insect populations, I also performed a laboratory bioassay on insect performance using leaves collected from the same field-grown plants. In mid-July I removed the youngest emerging leaf from each plant, inserted the petiole in a water-pick, and placed the leaf in a petri dish inside of a growth chamber. Second-instar beet armyworm larvae, *S. exigua*, were ordered from a biological supply company (Benzon Research, Inc., Carlisle, PA) and a single larva was added to each petri dish (n=75 control, n=74 nematode root herbivory). Initial caterpillar weights were recorded and caterpillars were weighed again after four days of growth. Relative growth rate was then calculated as $(w_f - w_i)/w_i/t$, where w_f = final weight, w_i = initial weight, and t = time. Caterpillar growth rates were compared between control and root herbivory plants using a t-test (Proc Glm).

Results

Patterns of insect-nematode co-occurrence – field survey

Plant-parasitic nematodes tended to be positively associated with leaf-chewing insects (Fig. 5.1A). Overall, tobacco-feeding nematodes (pooled total) were 41% more abundant in the rhizosphere of insect-defoliated plants compared with undamaged control plants ($F_{1,78} = 7.01$, $P = 0.0098$). Although there were consistent trends for the three most widely-distributed nematode genera to increase in abundance when associated with insect-defoliated plants, this association was marginally significant for *Tylenchorhynchus* ($F_{1,78} = 3.33$, $P = 0.0720$) and non-significant for *Pratylenchus* ($F_{1,78} = 1.00$, $P = 0.3202$) and *Xiphinema* ($F_{1,78} = 0.15$, $P = 0.7040$; Fig. 5.1A).

Sap-feeding aphids, however, showed the opposite pattern exhibiting mostly negative associations with nematode populations (Fig. 5.1B). The ectoparasitic nematode *Tylenchorhynchus* was less abundant in the rhizosphere of aphid-infested plants compared with control plants ($F_{1,15} = 8.71$, $P = 0.0099$), whereas the density of the migratory endoparasite *Pratylenchus* was unaffected by aboveground aphid herbivory ($F_{1,15} = 0.01$, $P = 0.9179$). The total phytoparasitic nematode community (comprised primarily of *Tylenchorhynchus* and *Pratylenchus*, but also including *Helicotylenchus*, *Xiphinema*, and *Hoplolaimus*) was less abundant beneath aphid outbreak plants, an effect that was marginally significant ($F_{1,15} = 3.91$, $P = 0.0666$).

Impact of foliar insect herbivory on belowground nematode population growth

The nematode communities sampled in 2006 and 2007 were very similar in taxonomic composition, despite occurring in different fields and years. I identified the following genera of plant-parasitic nematodes, along with their overall representation (i.e., presence/absence) among samples: *Helicotylenchus* (91.45%), *Tylenchorhynchus* (82.91%), *Xiphinema* (44.17%), *Pratylenchus* (29.06%), *Hoplolaimus* (23.33%), *Trichodorus* (9.17%), and *Meloidogyne* (0.85%). Because *Tylenchorhynchus* and *Helicotylenchus* were the only two genera that were consistently present in field cages, I restricted my statistical assessment to these nematodes. Neither hornworm caterpillars ($F_{1,69} = 0.04$, $P = 0.8415$) nor aphids ($F_{1,69} = 0.88$, $P = 0.3513$) affected the population growth of *Tylenchorhynchus* (Figs. 5.2A and 5.2B). However, caterpillar herbivory increased the population growth of *Helicotylenchus* ($F_{1,72} = 4.49$, $P = 0.0375$), whereas aphids had no impact ($F_{1,72} = 0.28$, $P = 0.6002$; Figs. 5.2C and 5.2D). The statistical interaction between caterpillar and aphid herbivory was non-significant for both nematode genera.

Effects of nematode root herbivory on aboveground insect populations

Root herbivory did not affect the density of tobacco hornworm eggs ($\chi^2 = 0.06$, $df = 1$, $P = 0.8031$) or the abundance of hornworm larvae ($\chi^2 = 2.98$, $df = 1$, $P = 0.0842$; Fig. 5.3A). *Epitrix* flea beetles, however, were more abundant on plants with elevated root herbivory ($\chi^2 = 4.36$, $df = 1$, $P = 0.0369$; Fig. 5.3A). Levels of foliar damage from chewing insects were no different on nematode-inoculated vs. control plants, as measured by the number of damaged leaves ($F_{1,147} = 0.55$, $P = 0.4603$; Fig. 5.3B) and overall percent defoliation ($F_{1,147} = 0.74$, $P = 0.3919$). Although there was a trend for beet

armyworm caterpillars, *S. exigua*, to grow more rapidly on plants with root herbivory, this effect was not significant ($F_{1,147} = 2.35$, $P = 0.1278$; Fig. 5.3C).

Nematodes reduced the abundance of aphids when populations were beginning to develop in July ($F_{1,148} = 3.82$, $P = 0.0524$; Fig. 5.3D). However, this effect was not detected when aphid populations approached peak densities in August ($F_{1,147} = 0.49$, $P = 0.4845$; Fig. 5.3E).

Discussion

Plants are known to mediate interactions in phytophagous insects (Van Zandt and Agrawal 2004, Viswanathan et al. 2005, Denno and Kaplan 2007, Kaplan and Denno 2007), but the community-wide effects of induced responses for lesser studied phytoparasitic taxa are only beginning to emerge (see Ohgushi et al. 2007). In particular, plant-parasitic nematodes, despite their abundance in terrestrial ecosystems and impact on plant productivity and diversity, have only recently been integrated into such ecological studies (Wardle et al. 2004b, van Dam et al. 2005, De Deyn et al. 2007, Wurst and van der Putten 2007, Kaplan et al. in review).

Using large-scale field surveys and manipulations of above- and belowground insect and nematode herbivory I demonstrate that herbivore-induced plant responses link root-feeding nematodes with foliar-feeding insects. Notably, the outcome of insect-nematode interactions was highly contingent upon insect feeding guild. Positive plant-mediated interactions predominated when chewing insects (e.g., caterpillars) were associated with tobacco leaves (Figs. 5.1A, 5.2C, 5.2D, and 5.3A), whereas negative interactions occurred with interactions involving sap-feeding insects (e.g., aphids; Figs.

5.1B and 5.3D). This guild-based divergence was largely consistent across trials conducted during different years, at different field sites, and involving several insect taxa. It is also noteworthy that we documented similar outcomes in recent experimental studies on insect and nematode performance in controlled greenhouse and laboratory environments. For example, root herbivory by *M. incognita* improved the performance (larval weight gain) of generalist caterpillars (*S. exigua* and *Trichoplusia ni*), and foliar herbivory by chewing insects (*M. sexta* and *S. exigua*) similarly increased the fecundity of root-galling nematodes (Kaplan et al. in review). Additionally, aphid growth rates and fecundity were lower on plants with nematode root herbivory (Kaplan et al. unpublished manuscript). Thus, guild-specific facilitative and competitive dynamics appear to characterize insect-plant-nematode interactions in the tobacco system. Below I discuss the diverse mechanisms that likely mediate these opposing responses.

Mechanisms underlying insect-plant-nematode interactions

Positive effects of nematode root herbivory on aboveground chewing insects

In earlier work on plant-mediated interactions between root- and shoot-feeding insects, Masters et al. (1993) proposed a model whereby root herbivory induces plant water stress leading to higher concentrations of soluble nitrogen in leaves and improved performance of foliar herbivores. Although I find that nematode root herbivory benefits leaf-chewing insects, the mechanism underlying this interaction is unlikely to involve water stress. I measured plant physiological responses that would be indicative of water stress (e.g., stomatal conductance) on nematode-inoculated plants and found no differences (I. Kaplan, unpublished data). Also, the C:N ratio of tobacco leaves increases

when nematodes gall roots, and thus the nutritional quality of tobacco leaves is reduced by root herbivory (I. Kaplan, unpublished data).

Instead, I emphasize the importance of secondary plant chemistry in nematode-induced facilitation of aboveground chewing insects. Tobacco alkaloids (i.e., nicotine) have toxic properties when ingested by herbivores and thus defend leaves against defoliators (Steppuhn et al. 2004). Importantly, tobacco alkaloids are synthesized in roots and subsequently translocated aboveground (Dawson 1941). This allows for the possibility that root herbivory reduces the efficacy of an important foliar defense. In previous work I found that nematode root herbivory interferes with the constitutive and inducible foliar expression of tobacco alkaloids (Kaplan et al. in press). Moreover, I also found that nematode-induced caterpillar facilitation only occurred on nicotine-producing tobacco plants, and not on nicotine-deficient mutant plants (Kaplan et al. in review). The fact that tobacco hornworms were unaffected by root herbivory in field experiments (Figs. 5.3A and 5.3B) is also consistent with an alkaloid-based mechanism. Hornworms can effectively detoxify and excrete alkaloids and therefore are far less sensitive to nicotine in their diet when compared with polyphagous insects (Wink and Theile 2002).

Positive effects of leaf-chewing insects on belowground nematodes

One of the two plant-parasitic nematode genera (*Helicotylenchus*) that was widely distributed in experimental fields exhibited consistently higher population growth rates when associated with plants that experienced insect defoliation (Figs. 5.2C and 5.2D). I have similarly documented higher fecundity of root-knot nematodes (*M. incognita*) on insect-defoliated plants in greenhouse trials. Although leaf-chewing insects do not affect

the secondary chemistry of tobacco roots (Kaplan et al. in press), foliar herbivory may induce changes in the source-sink dynamics of plant nutrients that benefit nematode root herbivores. Recent studies demonstrated an increase in the sink strength of roots when leaves were induced using either jasmonic acid (Babst et al. 2005) or caterpillar regurgitant (Schwachtje et al. 2006). Thus, foliar herbivory may elicit greater allocation of plant nutritional resources belowground, which may in turn benefit nematodes. Gall-forming herbivores, such as *M. incognita*, are known to benefit from increases in sink strength (Rehill and Schult 2003, Denno and Kaplan 2007). It is less clear if non-galling nematodes such as *Tylenchorhynchus* would similarly benefit via the same mechanism.

Negative interactions linking sap-feeding insects and phytoparasitic nematodes

Nematodes were less abundant on plants with aphid outbreaks (Fig. 5.1B) and early-season aphid population densities were lower on plants with enhanced root herbivory (Fig. 5.3D). These results also correspond with lower aphid performance on nematode-inoculated plants in greenhouse trials (I. Kaplan, unpublished manuscript). Moreover, a recent study on *Plantago lanceolata* documented lower aphid fecundity on plants attacked by the nematode *Pratylenchus penetrans* (Wurst and van der Putten 2007). Because the success of aphids is highly reliant on the nutritional quality of their food, gall-forming nematodes may negatively affect sap-feeders by attracting plant resources towards roots, thereby reducing aboveground allocation. However, given that aphids were negatively correlated with non-galling nematode taxa (i.e., *Tylenchorhynchus*), other mechanisms may contribute to such competitive dynamics. Although induced defenses are more often implicated in plant-mediated interactions

involving chewing insects (Denno and Kaplan 2007), it is noteworthy in this case that the same *Mi-1* gene that confers resistance to root-knot nematodes also provides resistance to aphids (Rossi et al. 1998, Vos et al. 1998). Moreover, recent evidence suggests that plant perception of and responses to sap-feeding insects are similar to pathogenic microorganisms (Kaloshian and Walling 2005). Thus, aphids and nematodes may be eliciting comparable defensive pathways in plants.

Conclusions

A large fraction of the earth's biodiversity consists of organisms associated with living plants. These consumers typically live and subsist on plant tissues, but they rarely kill their host (Ohgushi et al. 2007). Instead, their feeding behavior induces changes in plant phenotype (e.g., defense) that often render the plant more or less resistant to subsequent attack (Karban and Baldwin 1997). As a result, plants can mediate indirect interactions among a diverse array of consumers. However, the relative importance of such indirect, plant-mediated effects will be directly proportional to the within-plant spatial dynamics of induced responses. In other words, systemically-induced responses that alter the quality of all (or most) plant tissues are likely to have large-scale effects, whereas locally-induced responses (i.e., those that are only expressed in the damaged tissue) have limited potential for affecting the herbivore community at large.

Leaf-root connections represent the broadest spatial distance linking multiple plant parts. Yet, despite the spatial-separation that is characteristic of plant leaves and roots, I documented ecologically-relevant interactions between aboveground insect and belowground nematode communities. Notably, these indirect effects were discernable

under realistic field conditions where substantial heterogeneity in biotic and abiotic variables is commonplace. A major challenge for future studies will be to integrate the diverse taxa of consumers beyond insects to understand how plant responses to biotic attack serve to structure their associated community of above- and belowground parasites.

Figure Legends

Figure 5.1. The abundance of plant-parasitic nematodes in the rhizosphere of plants with naturally-occurring aboveground insect herbivory. Nematode populations were compared beneath (A) insect-defoliated vs. undamaged plants, and (B) aphid-infested vs. control (low aphid density) plants (means + SE). n.s. = non-significant.

Figure 5.2. The impact of foliar herbivory by leaf-chewing (caterpillar: *Manduca sexta*) and sap-feeding (aphid: *Myzus persicae*) insects on the population growth [$\log(N_F/N_0)$] of two genera of phytoparasitic nematodes (means + SE). Aboveground insect herbivory was manipulated in field cages over two years and belowground nematode populations were monitored, including *Tylenchorhynchus* in (A) 2006, and (B) 2007, and *Helicotylenchus* in (C) 2006, and (D) 2007. N_F = final population density, N_0 = initial population density.

Figure 5.3. The effects of experimentally-imposed nematode root herbivory on aboveground insect herbivores, including (A) the density of tobacco hornworm (*M. sexta*) eggs and larvae, and *Epitrix* flea beetle adults, (B) the number of leaves damaged by chewing insects, (C) the relative growth rate (RGR) of *S. exigua* larvae, (D) early-season aphid populations, and (E) late-season aphid populations (means \pm SE). Odds ratios quantify the abundance of herbivores in the root herbivory treatment relative to control plants. Thus, values greater or less than one

indicate that insects are more or less abundant, respectively, on plants incurring root herbivory. An asterisk denotes statistical significance ($P < 0.05$).

Figure 5.1

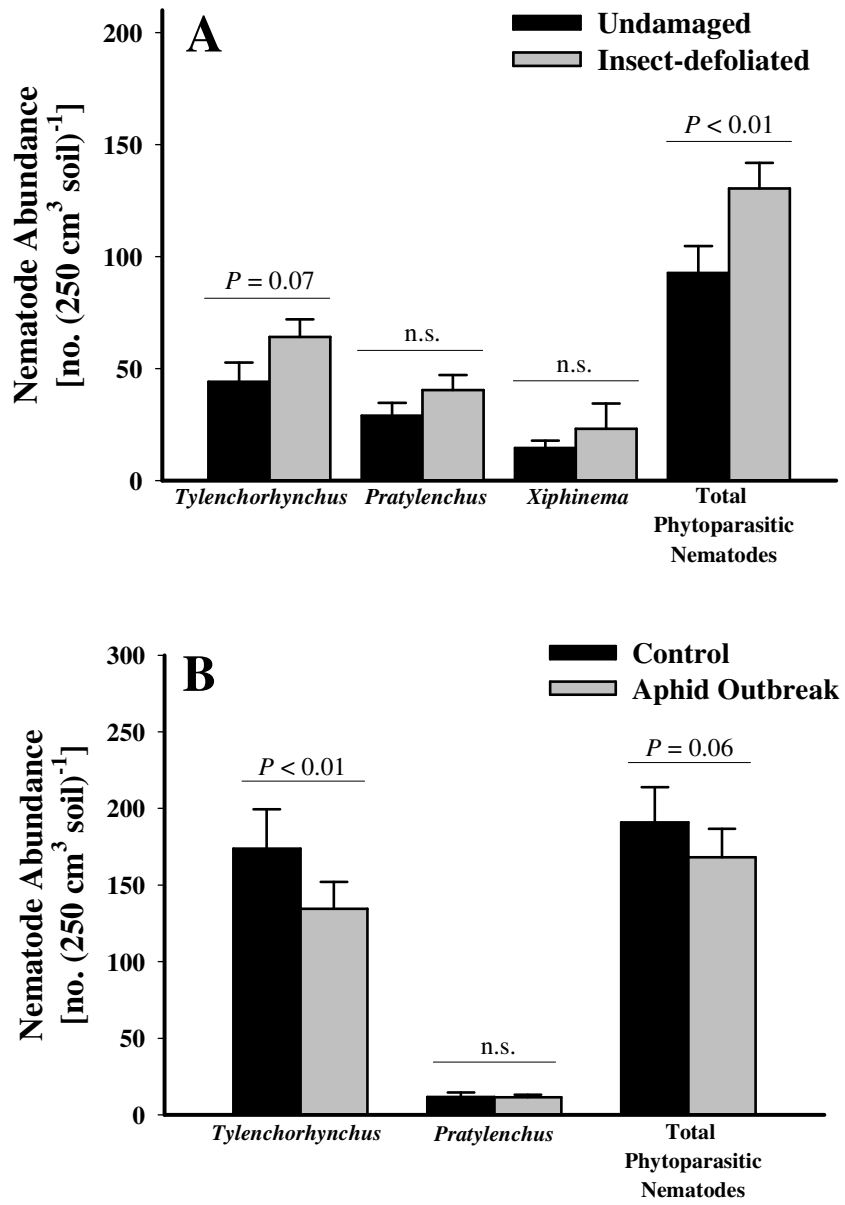


Figure 5.2

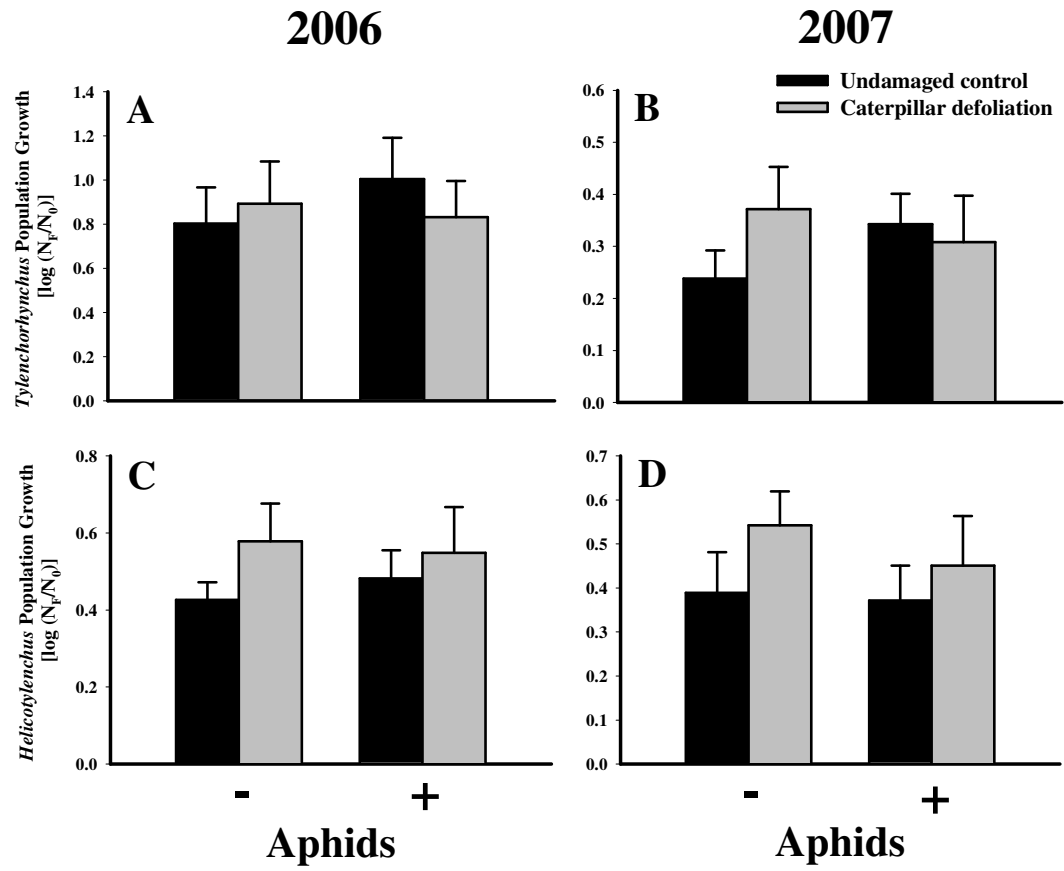
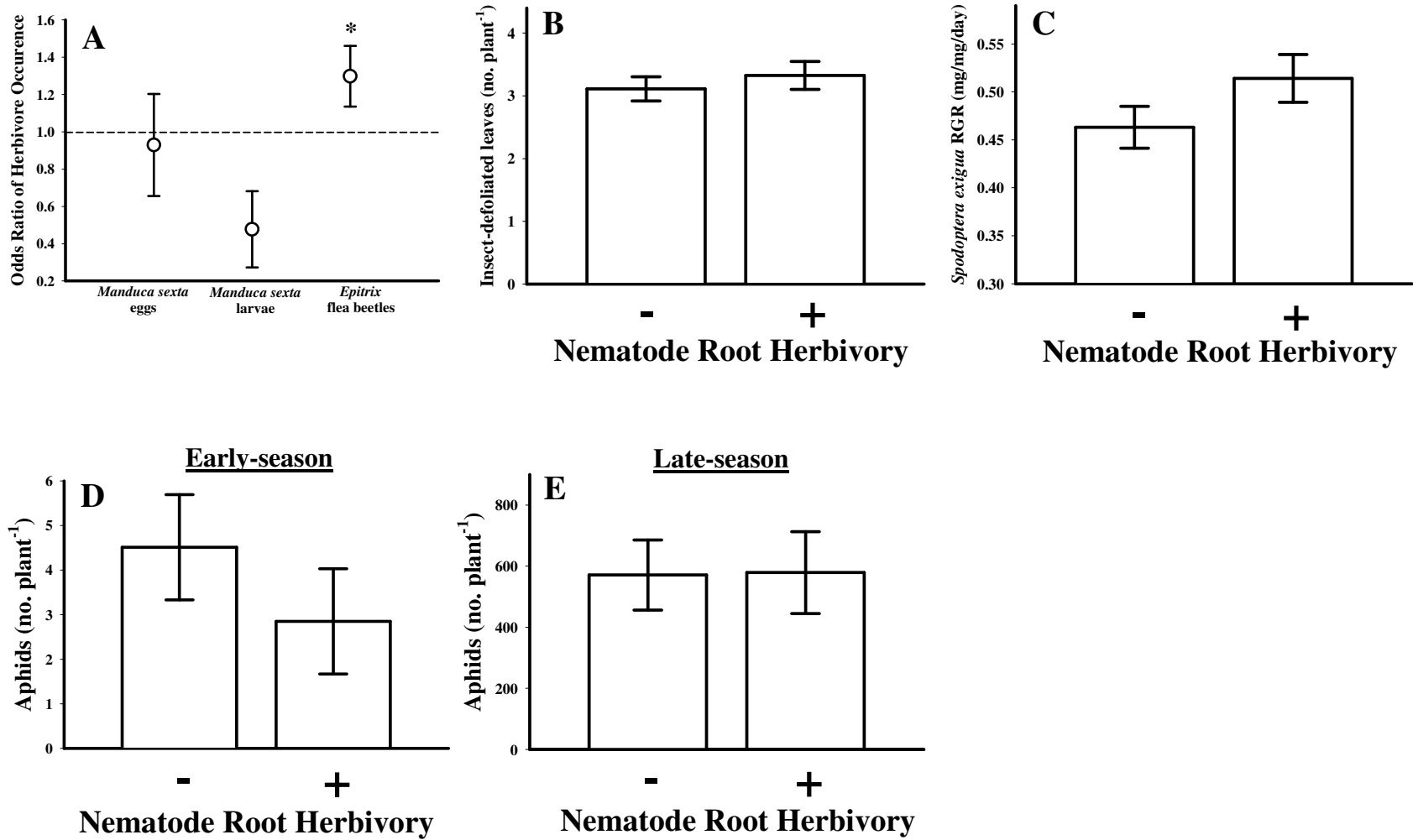


Figure 5.3



Appendix A: Studies used in Meta-Analyses

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