Field evidence for indirect interactions between foliar-feeding insect and root-feeding nematode communities on *Nicotiana tabacum*

IAN KAPLAN, SANDRA SARDANELLI and ROBERT F. DENNO Department of Entomology, University of Maryland, College Park, Maryland, U.S.A.

**Abstract.** 1. As herbivory often elicits systemic changes in plant traits, indirect interactions via induced plant responses may be a pervasive feature structuring herbivore communities. Although the importance of this phenomenon has been emphasised for herbivorous insects, it is unknown if and how induced responses contribute to the organisation of other major phytoparasitic taxa.

2. Survey and experimental field studies were used to investigate the role of plants in linking the dynamics of foliar-feeding insects and root-feeding nematodes on tobacco, *Nicotiana tabacum*.

3. Plant-mediated interactions between insects and nematodes could largely be differentiated by insect feeding guild, with positive insect–nematode interactions predominating with leaf-chewing insects (caterpillars) and negative interactions occurring with sap-feeding insects (aphids). For example, insect defoliation was positively correlated with the abundance of root-feeding nematodes, but aphids and nematodes were negatively correlated. Experimental field manipulations of foliar insect and nematode root herbivory also tended to support this outcome.

4. Overall, these results suggest that plants 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.

**Key words.** Aboveground–belowground interactions, community ecology, indirect effects, induced plant responses, *Nicotiana tabacum*, phytoparasitic nematodes, root herbivory.

Introduction

Perhaps the most pervasive opportunity for two 'separate' communities to influence one another occurs at the soil–air interface where plants serve as a conduit linking the dynamics of above- and belowground biota (van der Putten et al., 2001; 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 & van Dam, 2005; Kaplan et al., 2008a). Oftentimes, induced responses are transmitted via plant hormones (e.g. jasmonic acid) that elicit systemic changes in anti-herbivore resistance traits (van Dam et al., 2003; Bezemer & van Dam, 2005). Thus, herbivory in leaves or roots can induce whole-plant changes in allelochemistry (Bezemer et al., 2004; Kaplan et al., 2008a), resulting in indirect effects on co-occurring species (Bezemer et al., 2003; Soler et al., 2005, 2007; van Dam et al., 2005). In addition, many induced secondary plant chemicals (e.g. phenolics) have broad-spectrum efficacy and deter a wide range of consumers (Karban & Baldwin, 1997). Therefore, dissimilar organisms (e.g. insects, nematodes, fungi) with diverse feeding styles may nonetheless affect one another (Stout et al., 2006; Kaplan & Denno, 2007). Last, induced responses to herbivory have recently 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; Kaplan et al., 2008b).
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 m⁻²) on many plant species where they impair the translocation of water/nutrients and thus limit primary production (Stanton, 1988; Ingham & Detling, 1990). Phytophagous insects are the aboveground counterparts to nematodes, where they are well recognised for their detrimental effects on plants in natural and managed habitats (Marquis, 1992). Both nematodes and insects are present in virtually all terrestrial ecosystems, yet potential interactions between these groups of plant parasites are poorly documented. Mounting evidence, however, suggests that insect–plant–nematode interactions may indeed occur (e.g. Wardle et al., 2004b; van Dam et al., 2005; De Deyn et al., 2007; Wurst & van der Putten, 2007).

As most of the above-cited 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 primarily on unidirectional interactions (i.e. the impact of nematodes on insects, or vice versa), thus ignoring reciprocal dynamics. We 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 such above–belowground linkages can be discerned under realistic field conditions despite widespread heterogeneity in biotic and abiotic variables, is a critical step in this emerging ecological field. Indeed, Johnson et al. (2008) recently reviewed the literature on interactions between above- and belowground herbivory and identified the following two deficiencies requiring greater research: (1) moving beyond simple pairwise species interactions that currently dominate the literature and incorporating more complex communities, and (2) transitioning studies from laboratory-based investigations to field experiments.

Study system and mechanistic hypotheses

The phytophagous insect and nematode communities associated with the agricultural crop plant tobacco, *Nicotiana tabacum*, was used as the study system. 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* (Lepidoptera: Sphingidae) is the most abundant and damaging insect, but *Epirrita* flea beetles (Coleoptera: Chrysomelidae) often reach high densities as well. Moreover, a variety of sub-dominant generalist caterpillars (i.e. *Heliothis virescens*, *Spodoptera exigua*; Lepidoptera: Noctuidae) are also present. Of the sap-feeders, the aphid *Myzus persicae* (Hemiptera: Aphididae) is the dominant species, out-numbering all other sap-feeding insects by several orders of magnitude (I. Kaplan, unpublished data). 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 & Lucas, 1984). However, several genera of tobacco-feeding ectoparasites were consistently present in many of our fields.

Although mechanistic hypotheses have been proposed for interactions involving root- and shoot-feeding insects (e.g. Masters et al., 1993), more recent studies on induced plant defences contradict the generality of these earlier predictions (Bezemer & van Dam, 2005). Moreover, interactions between above- and belowground consumers often vary widely between being positive, negative, and neutral across different plant and herbivore systems (Johnson et al., 2008). Thus, we rely primarily on our earlier performance assays in greenhouse studies using the same combination of plants, insects, and nematodes to make predictions regarding the outcome of field trials. In prior work in the tobacco system, we found that root herbivory by *M. incognita* interferes with foliar nicotine expression (*M. incognita*), leading to improved performance of leaf-chewing insects on nematode-damaged plants (Kaplan et al., 2008b). Similarly, aboveground herbivory by *M. sexta* elicited a putative tolerance response, whereby plant nutritional reserves were preferentially allocated to roots, and thus caterpillars indirectly increased the fecundity of nematodes (*M. incognita*) sharing the same host plant (Kaplan et al., 2008b). Thus, we predict that leaf-chewing insects and root-feeding nematodes will positively affect the abundance of one another in field experiments. Unlike chewing insects, nematodes have been found to negatively interact with sap-feeding insects (Wurst & van der Putten, 2007), and preliminary investigations in the tobacco system confirms this to also be the case for aphids, *M. persicae*, and nematodes, *M. incognita* (I. Kaplan, unpublished data). Therefore, we predict that sap-feeding insects (aphids) and root-feeding nematodes will negatively affect the abundance of one another in field trials.

Materials and methods

Patterns of insect and nematode co-occurrence – field survey

To test for potential associations between above- and belowground herbivores, tobacco plots that were naturally colonised by insects and nematodes were surveyed to assess patterns of foliar and root herbivory. In 2006, insects and nematodes were surveyed in two adjacent plots (each plot ≈500 plants) at the Central Maryland Research and Education Center (Upper Marlboro, Maryland, U.S.A.). As nematodes are patchily distributed over small spatial scales, adjacent plots were used to minimise potential differences in nematode abundance and community composition. Separate plots were used to survey each insect feeding guild – one for chewing insects and one for sap-feeding aphids – due to logistical reasons associated with independently assessing the relationship between nematodes and each insect group. Both plots were cultivated with standard agronomic inputs of nitrogen, but were not irrigated or treated with insecticides.

In plot 1, we 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: (1) undamaged \((n = 36)\) or (2) insect-defoliated \((n = 44)\). Undamaged plants had virtually no evidence of herbivory \((< 1\% \text{ leaf area removed})\), whereas insect-defoliated plants received substantial damage from leaf-chewing insects \((20–60\% \text{ leaf area removed})\). Defoliation estimates were visually assessed by two separate observers, and the average of these two observations was used to separate plants into their respective groups. Other herbivorous insects \(\text{(e.g. aphids)}\) were not present on plants in either of the groups. Plants were distributed such that no two observations within each group were \(< 3 \text{ m}\) from one another, and often, the between-plant distances were far greater. Furthermore, plants in the two groupings were interspersed and thus, undamaged plants were frequently neighbouring defoliated ones. All plants were uprooted and the rhizosphere soil adhering to the roots was collected for nematode extraction.

In plot 2, we tested for an association between aboveground sap-feeding insects \(\text{(aphids)}\) and belowground nematode herbivory. Tobacco aphids \(\text{(M. persicae)}\) are late-season feeders and attain peak densities between late July and early August. Aphid outbreaks, however, are patchily distributed such that outbreak plants \(\ (> 10,000 \text{ aphids per plant})\) are often adjacent to plants with relatively few aphids \(\ (< 1000 \text{ individuals})\). Therefore, we visually surveyed and identified plants with aphid outbreaks and matched them with neighbouring plants possessing few aphids. As a result, plant groupings were blocked spatially within the plot \((n = 20 \text{ blocks})\). Plants that were defoliated by chewing insects were excluded so that the assessment isolates the relationship between aphids and nematodes. Soil samples were collected from the rhizosphere of plants and used to estimate nematode abundance.

Nematodes were extracted from soil \(\text{(each sample 250 cm}^3)\) using a modified version of the Baermann funnel technique. Briefly, soil samples were placed in water and subsequently poured onto nested sieves \(\text{(850-\mu m-pore sieve on top of a 45-\mu m-pore sieve)}\). The resulting nematodes were placed on Baermann funnels and collected after 48 h. All plant-parasitic nematodes present in the samples were identified to genus and counted.

Nematode counts were log transformed and \textit{ANOVA} was used to compare the abundance of each genus of plant-parasitic nematode beneath \(\text{(1) insect-defoliated vs. undamaged plants, and (2) aphid outbreak vs. non-outbreak (control) plants)}\) \textit{PROC MIXED}; all statistical analyses were performed using \textit{SAS}, Version 9.1). Only nematode genera present in \(\geq 50\%\) of samples were analysed 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 \(\text{(i.e. blocks)}\) were considered as a random effect.

Although the experiment tests for potential plant-mediated associations between insects and nematodes, other factors such as natural enemies can also affect herbivore community composition. As tobacco leaves possess a dense layer of glandular trichomes, the aboveground natural enemy community is somewhat depauperate. The primary enemies of tobacco aphids are larvae of \textit{Harmonia axyridis} \(\text{(Coleoptera: Coccinellidae)}\), whereas tobacco hornworms are primarily attacked by the parasitic wasp \textit{Cotesia congregata} \(\text{(Hymenoptera: Braconidae)}\) \(\text{(I. Kaplan, pers. obs.)}\). Plant-parasitic nematodes are consumed by a large and diverse group of soil organisms, ranging from predaceous nematodes to nematophagous fungi and mites.

\textbf{Impact of foliar insect herbivory on belowground nematode population growth}

A field cage experiment replicated over 2 years \(\text{(2006 and 2007)}\) was conducted to test the effects of foliar insect herbivory on the population growth of plant-parasitic nematodes. In 2006, a field was used that was previously cultivated in corn for at least five consecutive summers, and in 2007, a different field was used \(\text{(for logistical purposes)}\) that was cultivated in tobacco during the previous summer \(\text{(2006)}\) and asparagus in years prior to that. The two fields were located at the same general site and separated by approximately 1 km. Both fields had a sandy loam soil that was tilled prior to use, neither field was irrigated, and both fields were fertilised at a rate of 100 lb nitrogen ha\(^{-1}\).

In late May, we erected fine-mesh screen field cages \(\text{(3.7} \times 3.7 \times 2.1 \text{ m, length} \times \text{width} \times \text{height)}\) supported by polyvinyl chloride \(\text{(PVC)}\) frames, with 3 m separating each cage from neighbouring cages. Although cages inevitably impose different environmental conditions \(\text{(i.e. shading, temperature)}\) that may affect insect and nematode communities, no obvious differences were noted in the feeding behaviour of insects that were added to caged plants. In 2006, 14 total cages were constructed, whereas 15 cages were used in 2007. Aside from this minor difference in replication, the experimental protocol was virtually identical across the 2 years. Cages were large enough to enclose four tobacco plants and prevented insects from naturally colonising experimental plants. Tobacco seedlings were grown from seed in a greenhouse and transplanted into field cages in early June.

The presence of aboveground chewing and sap-feeding insects was manipulated in a \(\text{2} \times 2\) factorial design, resulting in one of four treatments that was randomly assigned to each seedling per cage: (1) control \(\text{(no herbivory)}\), (2) caterpillar, (3) aphid, and (4) caterpillar and aphid. The two dominant chewing and sap-feeding insects of tobacco, \textit{M. sexta} and \textit{M. persicae}, respectively, were used for the insect manipulations. Caterpillars were obtained from a locally available colony \(\text{(NC State University)}\), and aphids were collected from nearby tobacco fields. Insects were added to plants approximately 2 weeks after seedlings were transplanted into field cages \(\text{(mid June)}\). A single first-instar \textit{M. sexta} larva was added to ‘caterpillar-defoliated plants’, and 25 aphids were placed on ‘aphid-infested plants’. Cages were surveyed visually 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 \(\text{(i.e. plants in which caterpillars died and thus did not damage leaves)}\) were excluded from the analysis. On average, caterpillars defoliated two to three leaves per plant \(\text{(20–35}\%\text{ of the total leaf area)}\), 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 × 15 cm, width × 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 density associated with each treatment plant. Nematodes were sampled from the soil and not from the roots of plants, because ectoparasitic species dominated the community and endoparasites (e.g. Meloidogyne) were exceedingly rare. Furthermore, visual observations of roots from plants cultivated in these fields suggested that gall-inducing species were not present.

Nematode population growth was quantified as final density \( N_f \) divided by starting density \( N_i \). As this population growth estimate was not normally distributed, the variable was log transformed. 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, we manipulated root herbivory on field plants to test the reciprocal impact of nematodes on aboveground insect herbivores. A single plot 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 randomised design and thus, root herbivory plants were interspersed with control plants. In previous summers, this field was sampled extensively and population densities of plant-parasitic nematodes were low. Thus, it was assumed that control plants incurred minor levels of root herbivory.

The gall-forming nematode *M. incognita* was used as the focal root herbivore. Although *M. incognita* was absent or present at very low densities in the experimental fields, it is considered the dominant nematode parasite of tobacco worldwide and therefore, is the most ecologically relevant species in the context of root herbivory (Barker & Lucas, 1984). 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 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 & Lucas, 1984), and also corresponds with inoculum levels used in prior studies on *M. incognita*-tobacco interactions (see Kaplan et al., 2008a). As direct nematode inoculations onto field grown plants proved difficult in previous attempts, we first inoculated and grew tobacco seedlings in pots at a greenhouse. After 2 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. Galls were only detected on plants that received the early-season nematode treatment, and it can therefore be concluded that the inoculations were successful and that *M. incognita* did not occur on the roots of control plants.

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, foliar damage inflicted on control and experimental plants by leaf-chewing insects was assessed, including (1) the proportion of leaf area defoliated, and (2) the number of leaves with >50% leaf area damaged. We 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 the effect of nematode root herbivory on chewing insects was analysed 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 & 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 analyse 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 analysed 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 colonisation and damage by naturally occurring insect populations, we also performed a laboratory bioassay on insect performance using leaves collected from the same field-grown plants. In mid-July, we removed the youngest emerging leaf from each plant, inserted the petiole in a water-pick, and placed the leaf in a Petri dish inside 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 4 days of growth. Relative growth rate was then calculated as \( \frac{(w_f - w_i)w}{t} \), where \( w_f \) is the
final weight, \( w_i \) is the initial weight, and \( t \) is 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. 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. 1a).

Sap-feeding aphids, however, showed the opposite pattern exhibiting mostly negative associations with nematode populations (Fig. 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. We 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%). As *Tylenchorhynchus* and *Helicotylenchus* were the only two genera that were consistently present in field cages, the statistical assessment was restricted to these nematodes. Neither hornworm caterpillars (\( F_{1,109} = 0.04, P = 0.8415 \)) nor aphids (\( F_{1,109} = 0.88, P = 0.3513 \)) affected the population growth of *Tylenchorhynchus* (Fig. 2a,b). However, caterpillar herbivory increased the population growth of *Helicotylenchus* (\( F_{1,72} = 4.49, P = 0.0375 \)), whereas aphids had no impact (\( F_{1,72} = 0.28, P = 0.6002 \); Fig. 2c,d). 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 (\( \chi^2 = 2.29, d.f. = 1, P = 0.1301 \)) or the abundance of hornworm larvae (\( \chi^2 = 2.98, d.f. = 1, P = 0.0842 \); Fig. 3a). *Epitrix* flea beetles, however, were more abundant on plants with elevated root herbivory (\( \chi^2 = 4.36, d.f. = 1, P = 0.0369 \); Fig. 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. 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. 3c).

Nematodes reduced the abundance of aphids when populations were beginning to develop in July (\( F_{1,148} = 3.82, P = 0.0524 \); Fig. 3d). However, this effect was not detected.
when aphid populations approached peak densities in August ($F_{1,147} = 0.49, P = 0.4845$; Fig. 3e).

**Discussion**

Plants are known to mediate interactions in phytophagous insects (Van Zandt & Agrawal, 2004; Viswanathan et al., 2005; Denno & Kaplan, 2007; Kaplan & 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 ecological studies (Wardle et al., 2004b; van Dam et al., 2005; De Deyn et al., 2007; Wurst & van der Putten, 2007; Kaplan et al., 2008a,b).

Using large-scale field surveys and manipulations of above- and belowground insect and nematode herbivory, it was demonstrated that plants can mediate linkages between root-feeding nematodes and foliar-feeding insects. Importantly, such interactions were documented under realistic field conditions, and thus, the present study complements earlier studies that have largely been laboratory- or greenhouse-oriented investigations (Johnson et al., 2008). The observed patterns, however, were relatively stronger in our observational survey than in manipulative experiments. Moreover, many of the potential interactions were non-significant or only marginally so. This may be partly related to design limitations of the present study. For example, the root herbivory manipulation supplemented nematodes on field plants and thus, control plants likely received low levels of nematode root herbivory. In other cases, however, this reflects the underlying ecology of the tobacco system. The abundance of hornworm ($M. sexta$) eggs and larvae, for instance, were not affected by nematode presence. This is not entirely surprising, because nematode root herbivory benefits leaf-chewing insects by interfering with foliar nicotine (Kaplan et al., 2008b). Hornworms are known to detoxify and excrete alkaloids, and therefore are far less sensitive to nicotine in their diet when compared with other chewing insects (Wink & Theile, 2002).
Notably, the outcome of insect–nematode interactions appeared contingent upon insect feeding guild. Positive plant-mediated interactions predominated when chewing insects were associated with tobacco leaves, whereas negative interactions occurred with interactions involving sap-feeding insects. Similar outcomes have been documented in recent experimental studies on insect and nematode performance in controlled greenhouse and laboratory environments (Kaplan et al., 2008b). Thus, guild-specific facilitative and competitive dynamics may characterise insect–plant–nematode interactions in the tobacco system. However, because relatively few insect species represent chewers and sap-feeders (i.e. low species-level replication), it remains speculative whether this pattern truly represents a guild-based divergence. Additional studies in other systems are needed to confirm whether such guild differences signify broader patterns or are unique to the tobacco system.

A recent study on Plantago lanceolata also documented negative aphid–nematode interactions with lower aphid fecundity on plants attacked by the nematode Pratylenchus penetrans (Wurst & van der Putten, 2007). Although induced defences are more often implicated in chewing insects (Denno & 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). Moreover, recent evidence suggests that plant perception of, and responses to sap-feeding insects are similar to pathogenic micro-organisms (Kaloshian & Walling, 2005). Thus, aphids and nematodes may be eliciting comparable defensive pathways in plants.

Patterns with leaf-chewing insects are less consistent. Nematode root herbivory has been found to have negative (van Dam et al., 2005), positive (Alston et al., 1991), and no effects (Carter-Wientjes et al., 2004) on herbivorous insects. Caterpillar damage, however, has consistently benefited the performance of nematodes sharing the same host plant (Russin et al., 1989, 1993; Alston et al., 1993).

**Conclusions**

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, the present study documented ecologically relevant interactions between aboveground insect and belowground nematode communities. Notably, these indirect effects were discernible under 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 below-ground parasites.

**Acknowledgements**

We thank Brian Crawford, Maggie Douglas, Kevin Conover, and Mark Spicknall for their help in establishing and maintaining field experiments.
References


Accepted 28 September 2008
First published online 15 December 2008