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Plant-mediated multitrophic interactions between aboveground and belowground insects

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KEY WORDS
***Brassica nigra*, Brassicaceae, *Delia radicum*, Diptera, Anthomyiidae, *Cotesia glomerata*, Braconidae, associational resistance, indirect defence, *Pieris brassicae*, Pieridae, Lepidoptera**

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It is well documented that plants can act as vertical communication channels between below- and aboveground plant-feeding insects: root-feeders induce changes in biomass and chemistry of the plant's shoot, and these changes can influence survival, growth and development of leaf-feeders. The main aim of my project was to explore whether and how this interaction between spatially separated insects is restricted to plant feeders (root- and leaf-feeding insects), or can be extended to higher trophic levels, such as insect parasitoids. My model system consisted of the wild cruciferous plant *Brassica nigra* (first trophic level), belowground the specialist root herbivore *Delia radicum* (second level) and its parasitoid *Trybliographa rapae* (third level), and aboveground the specialist leaf-feeder *Pieris brassicae* (second level) and its parasitoid *Cotesia glomerata* (third level) plus the hyperparasitoid *Lysibia nana* (fourth level). The first experiments demonstrated that root- and leaf-feeders negatively affect each other's performances by increasing the levels of toxins in the host plant, and these negative effects were not restricted to the herbivore level, but transmitted to the parasitoid and even the hyperparasitoid. It was remarkable that the parasitoid was the most affected trophic level. I found out that the parasitoid female avoids to parasitise hosts on root-infested plants when hosts feeding on root-uninfested plants were readily available, probably based on changes in the volatile blend of the plant, triggered by the root-feeders. It even appeared that root-feeders can influence the foraging efficiency of parasitoids, even when the leaf-feeders (i.e., the parasitoid's host) and the root-feeders are not feeding on the same plant, but on conspecific plants within the same habitat. Finally, in a field experiment the two most abundant leaf-feeding insects preferred to feed and oviposit on root-uninfested plants. Hence, behavioural decisions in naturally occurring populations of leaf-feeding insects can be influenced by root-feeders under field conditions, where a multitude of interactions take place simultaneously. Interaction effects between above- and belowground organisms clearly are not limited to insects directly associated with the plant, but such interactions operate in a complex web of communication among various trophic levels.

Introduction

In terrestrial ecosystems, indirect interactions occur when a third species modifies the interaction between two species (Wooton 1994). Such indirect interactions can be extremely important in strengthening or weakening the direct interactions within food webs (Schmitz *et al.* 2000). Indirect interactions can also connect species that do not directly interact – for example, plants in the first trophic level and carnivores in the third trophic level – via foliar herbivores in the second trophic level. In terrestrial food webs, the study of indirect interactions has traditionally focused on organisms that share a common domain: either below or above the soil surface. Considering that plants develop both underground and aboveground, they potentially mediate indirect interactions between organisms that belong to these spatially separated domains.

In the last two decades it has indeed been shown that subterranean organisms that are intimately associated with the roots of terrestrial plants can influence the biomass and levels of primary and secondary compounds of aboveground plant parts (Bezemer & Van Dam 2005). This in turn can strongly influence the growth, development and survival of insects feeding on the shoot of the plant (Gange & Brown 1989,

NEV Dissertatieprijs 2008

Tijdens de 20e Nederlandse Entomologendag (Ede, 19 december 2008) is voor de allereerste keer de NEV Dissertatieprijs uitgereikt. Deze prijs bestaat uit een geldbedrag plus een oorkonde en wordt toegekend voor het beste proefschrift op het gebied van de entomologie, verdedigd aan een Nederlandse universiteit in het voorgaande jaar (1 september – 31 augustus).

De NEV Dissertatieprijs 2008 is uitgereikt aan Dr. Roxina Soler Gamborena, voor haar proefschift 'Plant-mediated multitrophic interactions between aboveground and belowground insects', op 30 oktober 2007 verdedigd aan de Wageningen Universiteit. Het werk is grotendeels uitgevoerd aan het Nederlands Instituut voor Oecologisch Onderzoek, te Heteren.

De jury was van oordeel dat dit proefschrift innovatief onderzoek van een hoog niveau laat zien. Het werk is sterk entomologisch getint, maar heeft een breder ecologisch belang door de inbedding in de community ecology theorie. De onderzoeksmethoden lopen uiteen van minutieuze chemische analyses tot aan gedragsobservaties aan een fors aantal soorten, in de context van kleinschalige laboratoriumexperimenten, maar ook ruimer opgezette experimentele veldproeven. De hoofdstukken van het proefschrift vormen een duidelijk samenhangend geheel.

During the 20th Annual Dutch Entomologists Meeting in Ede, December 19, 2008, the very first Netherlands Entomological Society (NEV) Dissertation Award was presented. This price comprises an amount of money and a certificate of appreciation, and is awarded for the best doctoral thesis in the field of entomology, defended at a Dutch university in the preceding year (1 September – 31 August).

The NEV Dissertation Award 2008 is presented to Dr. Roxina Soler Gamborena, for her thesis 'Plant-mediated multitrophic interactions between aboveground and belowground insects', defended at Wageningen University, on October 30, 2007. The work was largely done at the Netherlands Institute of Ecology, in Heteren.

The jury decided that this thesis demonstrates innovative research of a high level. The work is clearly rooted in entomology, but it has a much wider ecological interest because of its embedding in current community ecology theory. Research methods stretch from minute chemical analyses to behavioural observations of more than a few insect species, in the context of small scale laboratory experiments, but also of larger scale experimental field trials. The various chapters of the thesis contribute to a clear and coherent picture.

Masters *et al.* 1993, 2001, Masters 1995, Wardle 2002, Van der Putten *et al.* 2001, Wurst & Jones 2003, Bezemer & Van Dam 2005). Vice versa, aboveground herbivory has been shown to affect the development of belowground insect herbivores, when the shared host plant is previously exposed to insect attack in the shoot (reviewed in Blossey & Hunt-Joshi 2003), thereby creating plant-based functional links between soil-dwelling organisms and aboveground leaf-feeding insects.

The discovery that soil organisms, whose presence passes habitually unnoticed, can affect the functioning of plantherbivore communities aboveground, more recently raised the question whether also higher trophic levels such as carnivores could be influenced. Based on this challenging idea, at the end of 2003 I started my PhD project to study the possible consequences of the interactions between root- and leaf-feeding insects on the performance and behaviour of higher trophic levels, and the mechanisms that could mediate the interactions between such distant organisms. Figure 1 presents the model system used to address these questions.

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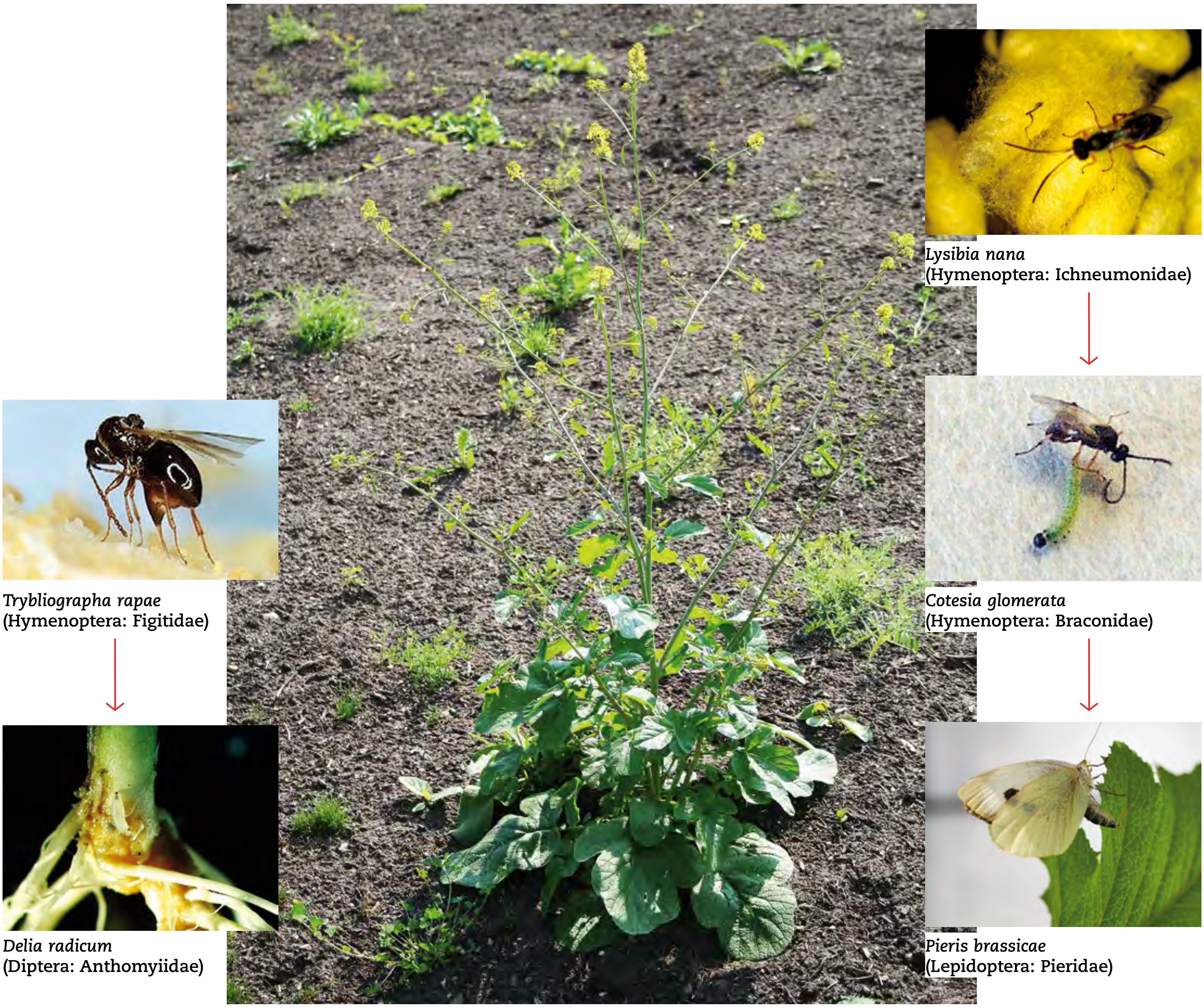


1. Model system. The system consist of: the wild cruciferous plant species *Brassica nigra* (Brassicaceae) (first trophic level), the specialist root herbivore species *Delia radicum* (Diptera: Anthomyiidae) (second trophic level, belowground), its natural enemy, the parasitoid *Trybliographa rapae* (Hymenoptera: Figitidae) (third trophic level, belowground), the specialist foliar-feeding herbivore species *Pieris brassicae* (Lepidoptera: Pieridae) (second trophic level, aboveground), its natural enemy the parasitoid *Cotesia glomerata* (Hymenoptera: Braconidae) (third trophic level, aboveground), and the secondary parasitoid (hyperparasitoid) *Lysibia nana* (Hymenoptera: Ichneumonidae) (fourth trophic level, aboveground). Photos: R. Soler, expect *T. rapae*: photographer unknown, *P. brassicae*: Sonja V. Schaper

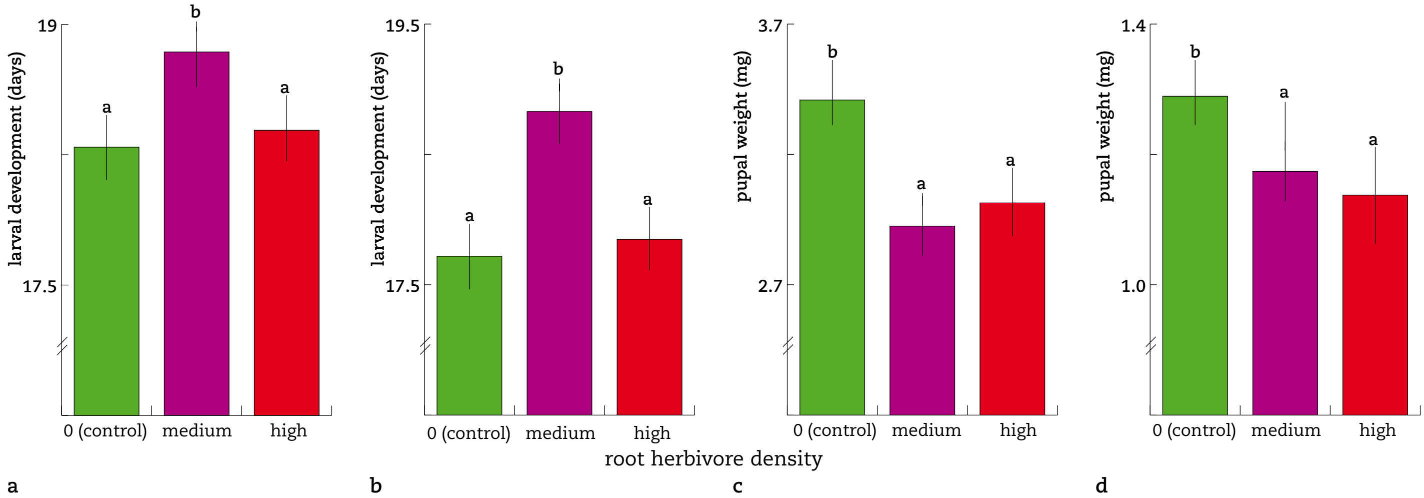
1. Modelsysteem, bestaande uit: de wilde kruisbloemige zwarte mosterd, *Brassica nigra* (1e trofische niveau), de gespecialiseerde worteletende koolvlieg, *Delia radicum* (2e trofische niveau), zijn ondergrondse natuurlijke vijand de parasitaire wesp *Trybliographa rapae* (3e trofische niveau), de gespecialiseerde bladeter *Pieris brassicae*, het grote koolwitje (2e trofische niveau, bovengronds), zijn (bovengrondse) natuurlijke vijand de parasitaire wesp *Cotesia glomerata* (3e trofische niveau, bovengronds), en de hyper-parasitaire wesp *Lysibia nana* (4e trofische niveau, bovengronds).

Herbivores in one domain affect the performance of herbivores and carnivores in the opposite domain

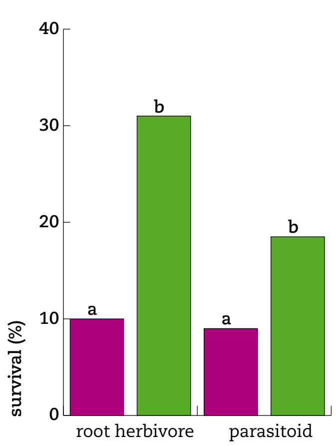
The first chapters of my thesis demonstrate that insects in the soil can strongly interact with herbivores and carnivores aboveground, and the other way around: foliar insects aboveground can influence the development of herbivores and carnivores in the soil. This discovery emphasises the importance of considering not only organisms directly associated to the plant roots and shoot, but also higher trophic levels when studying interactions between spatially separated organisms.

We found that root-feeding insects negatively affected the performance of the leaf-feeding insect (figure 2a), its parasitoid (figure 2b,c) and the hyperparasitoid (figure 2c). Shoot biomass, as well as the levels of primary plant compounds of the shoot of root-infested and root-uninfested plants, did not differ. However, the levels of sinigrin, the main secondary plant compound in the shoot, were more than folded in root-infested plants compared with the concentration recorded on root-uninfested plants, suggesting that the negative impact of root-herbivores on the performance of the aboveground insects is (at least partially) mediated by root-induced changes in the levels of secondary plant compounds of the shoot of the plant.

The density of root herbivores influenced the responses on the aboveground insects (figure 2). Overall, the effects of root herbivory on the development of the aboveground insects were stronger at 'medium' root herbivore density (5 *Delia* larvae/plant) than at 'high' density (20 larvae/plant). Plants exposed to 20 larvae probably experienced severe stress and were therefore unable to maintain an optimal defence system against herbivory.



2. Fitness correlates of the foliar herbivore (a), its parasitoid (b and c) and the hyperparasitoid (d) on root-uninfested plants (green bars), and on plants previously exposed for 13 days to medium (violet bar) and high (red bar) root herbivore densities.
2. Fitnessmaten (larvale ontwikkelingsduur en popgewicht) van de bladeter (a), zijn parasitoïd (b en c) en de hyper-parasitaire wesp (d) op planten waarvan de wortels niet zijn aangevreten (control), dan wel op planten waarvan de wortels gedurende twee weken zijn aangevreten door wortelknagers aanwezig in een matige (medium) of een hoge (high) dichtheid.



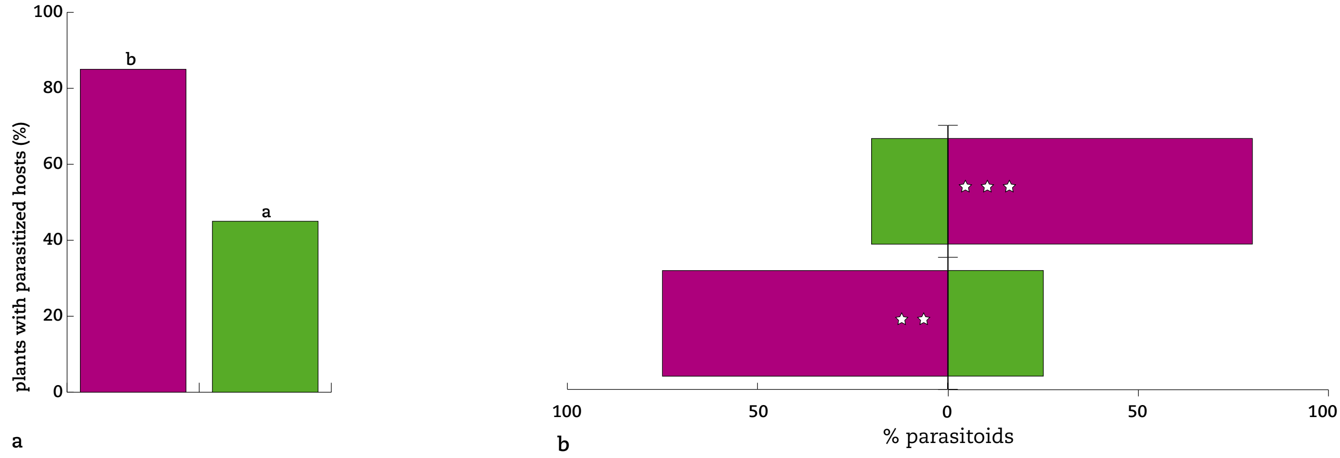
3. Fitness correlates of the root herbivore (a) and its parasitoid (b) on shoot-uninfested plants (violet bars) and on plants previously exposed to foliar herbivory by three first instar larvae during 3 days (green bars).
3. Fitnessmaat (overleving) van de worteleter (a) en zijn parasitoïd (b) op planten waarvan de spruit onaangetast is (paars), dan wel gedurende drie dagen is aangevreten door drie jonge larven (groen).

More attention should be paid to the effects of density of root herbivores or the severity of damage, when linking interactions between spatially separated insects, since this may profoundly impact the outcome of such studies. In naturally occurring populations of *Brassica nigra* in The Netherlands, as well as in *Brassica oleracea* (Brussels sprouts) crop fields, about 5-9 *Delia radicum* (Linnaeus) (Diptera: Anthomyiidae) larvae are often found in infested plants (R. Soler, pers. obs., summer 2006 and 2007; K. Winkler, pers. obs.). Selecting a naturally occurring system, combined with field-knowledge of the dynamics of this system, is an essential prerequisite for the design of controlled experiments, if we aim to obtain realistic answers to questions on how above- and belowground organisms interact via shared host plants.

Remarkably, in my studies the aboveground parasitoid was the trophic level most affected by the presence of root herbivores on the host plant, even though the foliar herbivores were feeding directly on the affected plants. This shows that changes in host plant quality induced by root herbivores become not only manifest one step up in the food chain, but can even be magnified at higher trophic levels. Based on these results – also considering that female parasitoids have evolved complex mechanisms to find their hosts and usually prefer to attack those that maximise the performance of their off-spring (Godfray 1994) – we then investigated whether such negative effects of root herbivores on the performance of parasitoid progeny might have consequences for host preference and oviposition behaviour of foraging parasitoids.

The reverse effect also takes place (figure 3). Leaf-feeding insects can negatively affect the performance of a root-feeding insect and its parasitoid. In this case also the interactions were mediated via induced changes in the levels of secondary plant compounds in the roots of the plant. Previous studies have shown that severe foliar damage by insects can strongly reduce the biomass of the plant roots, negatively influencing the growth and development of the belowground insects feeding on the limited root system (Masters *et al.* 1993, Tindall & Stout 2001, Blossey & Hunt-Joshi 2003). However, plants may respond in different ways when suffering from removal of tissue by insect herbivores, and the effects of herbivory on plants can exceed the simple loss of tissues. Damage from foliar herbivores, for example, can lead to a systemic increase in the expression of secondary plant compounds in the roots (Collantes *et al.* 1999), but most studies report no changes in root secondary chemistry following aboveground damage (Bezemer & Van Dam 2005). In our experiments, we exposed the plant shoot to only three young caterpillars for a short period of time to assure that the physical amount of foliar tissue removed was negligible, before growing the soil food-chain belowground. Therefore, we can conclude that minor damage caused by foliar-feeding insects can be sufficient to impact the performance of soil insects, by inducing a defence response in the plant resulting in increased levels of secondary plant compounds in the roots. This suggests that, even when the amount of damage in the plant shoot is negligible, plant-mediated effects of aboveground herbivory cannot be ignored in soil ecology.

In nature, virtually all plants experience at least some level of foliar damage. This can lead to altered performance of soil dwelling organisms over several trophic levels, which can ultimately result in changes in the composition and functioning of soil communities. Recently, soil ecologists have become increasingly aware of this, but the studies have focused predominantly on differences in nutritional quality between plant species (Bardgett & Shine 1999, Wardle 2002, De Deyn *et al.* 2004, Bezemer *et al.* 2006), and not on the effects of changes within a single plant species. There is an urgent need, therefore, for more studies that investigate how changes in root nutritional quality, induced by aboveground herbivory, affect belowground multitrophic interactions.



4. Percentage of plant-host complexes (plants with 10 larvae of the parasitoid host), 2 hours after releasing naïve female parasitoids in a large tent containing 13 root-uninfested (violet bar) and 13 root-infested (green bar) plants (a). Percentage of choices of the parasitoid, in a two-choice experiment between host-infested and host-uninfested plants (above) and between root-infested and root-uninfested plants (below) (b).
4. (a) Parasiteringssucces (% planten met geparasiteerde gastheren), 2 uur na loslating van naïeve vrouwelijke parasitaire wespen in een tent met 13 planten waarvan de wortels onaangetast zijn (paars) en 13 planten met aangevreten wortels (groen). (b) Verdeling van parasitaire wespen over planten met en zonder gastheren aanwezig (bovenste) en over planten met wel of niet aangetaste wortels (onderste).

Consequences for foraging parasitoids

Optimal foraging theory predicts that carnivores prefer to attack hosts and prey species that are most rewarding for them in terms of their fitness, measured as number and quality of their offspring (Stephens & Krebs 1986). Similarly, within a host species, parasitoid females are expected to select the most profitable individuals (Godfray 1994, Brodeur *et al.* 1998). The results of the study summarized in figure 4 indicate that, overall, the parasitoid exhibited a clear preference for (a) parasitizing and (b) searching for hosts on plants that had not been attacked by larvae of the root herbivore. In the earlier study summarized in figure 2 we report that the performance, i.e. the development time and pupal weight of the parasitoids, is negatively affected by foliar increases in phytotoxins induced by the root herbivore sharing the host plant. In this study we find that the parasitoid is able to recognize plants based on the presence or absence of root herbivores, and prefers to search and lay their offspring on hosts feeding on root-undamaged plants. Hence, we find a clear preference-performance pattern for the parasitoid. Theoretical models predict that oviposition decisions by parasitoid females lead to the selection of the most profitable host for their off-spring (Van Alphen & Visser 1990, Godfray 1994). These models have been exclusively based on aboveground model systems, whereas our results suggest that *Cotesia glomerata* (Linnaeus) (Hymenoptera: Braconidae) females are also able to exploit root-induced signals to evaluate and select the most suitable host for their off-spring.

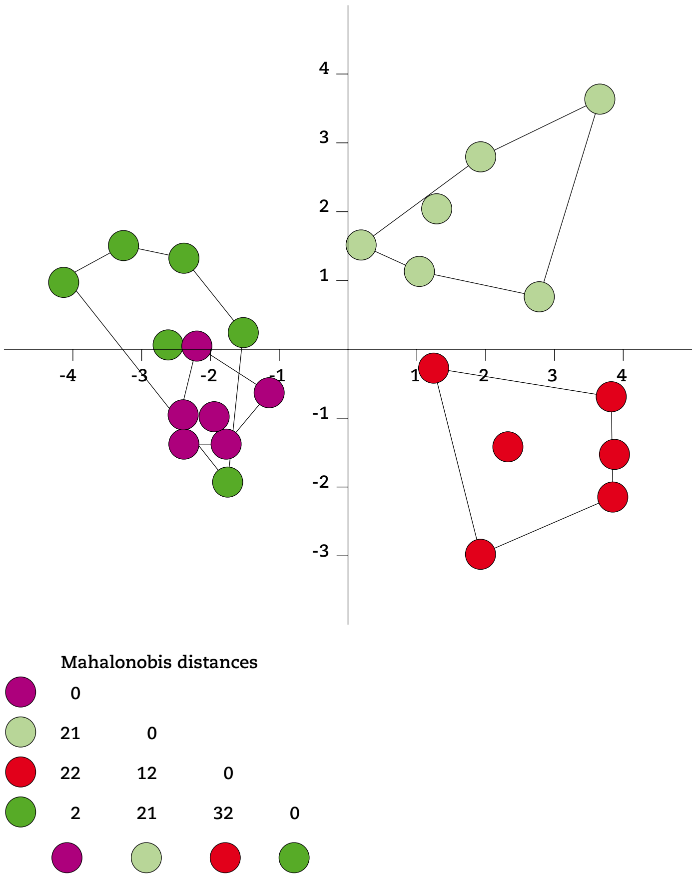
The underlying mechanism mediating the interactions between the root herbivores and the aboveground parasitoid points to changes in the volatile blend of the plant (figure 5). Overall, plants exposed to leaf herbivory were characterised by a high amount of beta-farnesene and dimethylnonatriene, and plants exposed to root herbivory were characterised by high amounts of dimethyl disulfide and dimethyl trisulfide. Beta-farnesene and dimethylnonatriene are volatile compounds reported to act as attractants for herbivorous and carnivorous insects (Fukushima *et al.* 2002, Ansebo *et al.* 2005). By contrast, dimethyl di- and trisulfide are reported to exert insecticidal neurotoxicity through mitochondrial dysfunction (Dugravot *et al.* 2003). Plants exposed to both root and leaf herbivory had volatile blends with higher levels of sulfides and lower levels of attractants compared with plants exposed to only leaf herbivory by the host. Consequently, the avoidance by the parasitoid of the plants exposed to root herbivory may be partly attributed to the higher amount of toxic volatiles combined with the lower production of attractants present in the blend of the root-damaged host-infested plants.

Contrary to this result, Poveda *et al.* (2005), using a related plant species (wild mustard, *Sinapis arvensis*) but with different above- and belowground insects, showed that aphid parasitoids were attracted to plants with aphids that were also exposed to root-feeding insects over conspecific root-undamaged plants. The authors did not investigate the effects of the soil dwelling organisms on the performance of the parasitoid offspring or the possible mechanisms mediating these interactions, thus making it difficult to compare their study with the results of my thesis. There are still relatively few studies including carnivores when examining interactions between spatially separated organisms (e.g., Gange *et al.* 2001, Rasmann & Turlings 2007), and thus general conclusions or generalisations cannot be drawn.

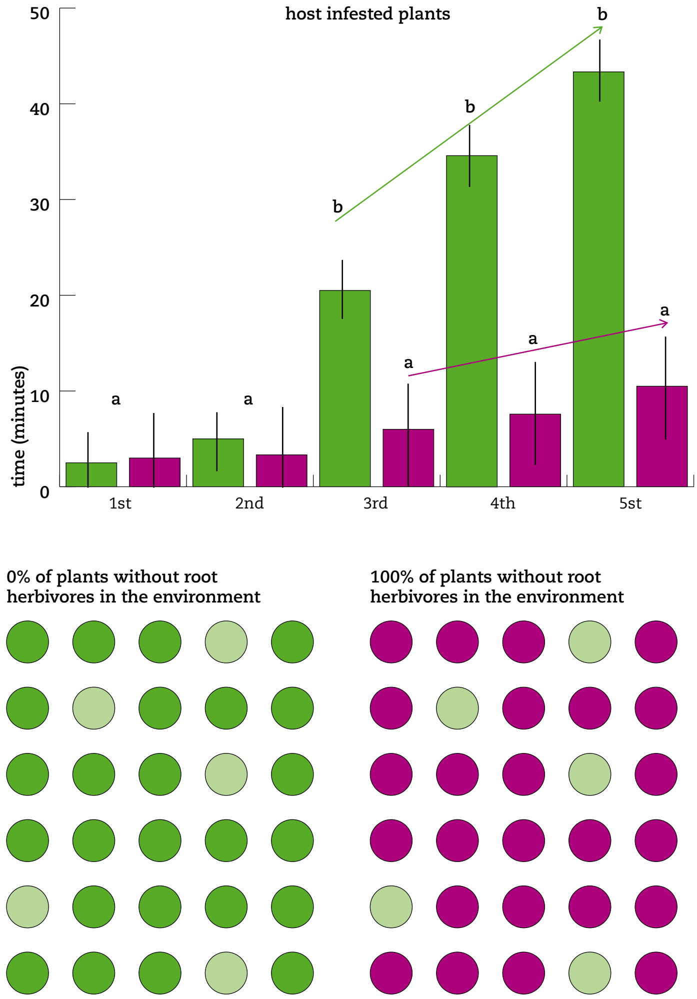
Assembling all these summarized results suggests the possibility of associational resistance between the spatially separated herbivores, where *Pieris brassicae* Linnaeus (Lepidoptera: Pieridae) gains protection from its parasitoid *C. glomerata* by association with its (belowground) competitor *D. radicum*. This phenomenon may have important consequences for the population dynamics among hosts and their specialised natural enemies. By protecting a proportion of the host population from parasitism, the non-host competitor provides the host with a refuge, via the presence of enemy-free space. For intimate host-parasitoid interactions, spatial refuges can be stabilising by preventing over-exploitation of the host population that would otherwise lead to local extinction of the host and ultimately the parasitoid (Begon *et al.* 1996). Further studies are needed to find out whether *P. brassicae* females prefer to oviposit on root-damaged plants, and whether associational resistance indeed takes place.

Habitat-mediated above-belowground interactions

Thus far, interactions between spatially separated organisms have been reported in situations where the ‘root’ and the ‘leaf’ organisms share the same plant (e.g., Bezemer *et al.* 2003, Guerrieri *et al.* 2004). We investigated whether the interaction between the root-feeding insect and the aboveground parasitoid could still take place if the root-feeding insect and the



5. Two-dimensional canonical discriminate analysis (CDA) plot of the volatile blends of plants exposed to root herbivory (red circles) or leaf herbivory (green circles), no herbivory (light green circles, control) or both types of herbivory (violet circles). Each point represents the volatile blend of a sampled plant. Below, Mahalonobis distances between the volatile blends are presented to quantify the relative (dis)similarities between the volatile blends of the four plant treatments, which measures the distance between treatment-cancroids in multivariate space.
5. Diagram waarin de gelijkenis staat weergegeven tussen de mengsels van vluchtige stoffen afkomstig van planten met aangevreten wortels (rode cirkels) of spruit (groen), onaangetaste planten (lichtgroen; controle), of met aangetast wortels en spruit (paars). Elke cirkel geeft het mengsel weer van een enkele plant. De getallen in de tabel geven een andere maat weer voor de gelijkenis: hoe groter het getal, des te groter het verschil tussen twee vergeleken mengsels.



6. Time spent by the female parasitoids to find five host-plant complexes in a large tent, within a surrounding environment consisting on 25 root-infested plants (violet bars, right scheme below), or within a surrounding environment consisting on 25 root-uninfested plants (green bars, left scheme below).
6. Invloed van de omgeving: tijd (in minuten) die vrouwelijke parasitaire wespen nodig hebben om vijf planten met gastheren te bezoeken in een grote tent, wanneer de planten omgeven zijn door 25 planten waarvan de wortels zijn aangevreten (paars; ruimtelijke situatie zoals rechtsonder), dan wel door 25 planten met onaangetaste wortels (groen; linksonder).

leaffeeding insect (i.e., the parasitoid host) were feeding on neighbouring conspecific plants. In an experiment conducted in a large tent, the proportion of neighbouring plants that were root-uninfested and root-infested was manipulated, and the time that parasitoids spent to locate five additional host-infested plants within the various surrounding environments was recorded. It appears that root herbivores can indeed influence aboveground host-parasitoid interactions via changes in the 'attractiveness' of surrounding conspecific plants (figure 6).

Complexes of herbivore species on a single plant can diminish the searching efficiency of foraging parasitoids, either by making the volatile blend of host-infested plants less reliable for searching parasitoids or by causing parasitoids to waste time searching on non-host larvae (McCann *et al.* 1998, Vos *et al.* 2001). We found that the presence of non-host (root) herbivores on surrounding plants enhanced the searching efficiency of the parasitoid, strengthening the host-parasitoid interaction. Because *B. nigra* plants exposed to root herbivory emit a volatile blend characterised by high levels of specific sulphur volatile compounds, and because these volatile cues seem to be used by the parasitoid females to identify and avoid plants with root herbivory (figures 4 and 5), we propose that the characteristic volatile blend emitted by plants exposed to root herbivory that surrounded the host-infested plants may have provided a more contrasting background than the blend emitted by nearby plants without root herbivory, thus benefiting parasitoids that are searching for hosts. In a habitat with plants of the same species, the differences in volatile blends between plants with and without hosts may not be clearly detectable at larger distances for searching parasitoids. Searching female parasitoids may therefore waste time on or near plants without hosts. In our study system plants without hosts, but exposed to root herbivory, repel searching parasitoids, and we suggest that in our experiment this has improved their ability to locate host plants by wasting less time on habitat plants without hosts. Here we provide evidence that female parasitoids can exploit qualitative characteristics of the surrounding environment, triggered by belowground insects, to maximise their searching efficiency. These results suggest that spatially separated organisms can interact in much more complex and indirect ways than has been proposed so far.

Aboveground-belowground root and foliar insect interactions in the field

Several studies have shown that above- and belowground insects can interact by influencing each others growth, development and survival when they are forced to feed on the same host-plant. In natural systems, however, insects can choose on which plants to oviposit and feed. We carried out a field experiment to determine whether root-feeding insects can influence feeding and oviposition decisions of naturally colonising leaf-feeding insects, placing in the field large groups of root-infested and root-uninfested *B. nigra* plants, and monitoring the naturally colonising populations of leaf-feeding insects over the course of a summer season.

We found that two of the most abundant leaf-feeding insects on *B. nigra*, the flea beetle *Phyllotreta* spp. and the aphid *Brevicoryne brassicae* (Linnaeus) (Hemiptera: Aphididae), significantly preferred to feed and oviposit on the shoots of root-uninfested plants rather than on the shoots of root-infested plants. Bearing in mind that the levels of foliar phytotoxins (glucosinolates) of root-infested *B. nigra* plants by *D. radicum* are more than twice as high as compared with root-uninfested plants, and that the specialised foliar herbivore *P. brassicae* develop suboptimally on these plants (figure 2), root-infested *B. nigra* plants may represent a suboptimal nutritional source for other species of foliar herbivores as well. Considering that *Delia* spp. commonly attack brassicaceous plants, foliar herbivores of these plants may be under strong selection pressure to avoid ovipositing or feeding on plants with *Delia* spp., particularly if this is correlated with a significant reduction in their fitness, and root-uninfested plants are readily available.

The observation that aboveground herbivores avoid plants that experience root herbivory implies the interesting possibility that the avoidance behaviour of *C. glomerata* parasitoids to root herbivore damaged plants is not necessarily related to fitness consequences for their offspring, but because, under natural conditions, hosts are less likely to be found on such plants.

General conclusion and future directions

An increasing number of studies published over the past 15 years has clearly shown that the soil system should not be seen as a black box, because the organisms that live there, such as decomposers, antagonistic root feeders, and mutualistists, can influence aboveground plant biomass and quality which, in turn, affect the performance of insects feeding aboveground (Van der Putten *et al.* 2001, Wardle 2002, Wardle *et al.* 2004, Bezemer & Van Dam 2005, Kaplan *et al.* 2008). The results of my thesis contributed to show, together with other contemporary studies (e.g., Omancini *et al.* 2001, Guerrieri *et al.* 2004, Bezemer *et al.* 2005), that the indirect interactions between spatially separated organisms are not restricted to the organisms directly sharing the host plant. Rather, herbivory can significantly influence the performance of higher trophic level organisms in the opposite compartment. Therefore, the interactive effects between above- and belowground organisms cannot be limited to organisms directly associated to the plant because they occur throughout a complex chain of multitrophic organisms. Ecological studies that claim that a better understanding of terrestrial ecosystems requires an aboveground-belowground approach because of the strong feedback between root and foliar organisms directly associated with the plant should emphasise the need to incorporate higher trophic levels.

The results of this thesis also show that 'hidden' root-feeding insects can influence the behaviour and searching efficiency of aboveground foliar herbivores and parasitoids. These results add to the empirical data generated from a number of studies (Omacini 2001, Masters *et al.* 2001, Gange *et al.* 2003, Wurst & Jones 2003, Guerrieri *et al.* 2004, Bezemer *et al.* 2005) and provide evidence that soil organisms can be a major component in the interplay of forces that shape community structure. Soil organisms, however, are still not included in mainstream ecological theory. It is time now to develop a theoretical framework that more broadly integrates 'aboveground-belowground organism interactions' into the field of ecology. This is, in my opinion, one of the most challenging issues in this field.

'It remains largely unstudied whether innate responses of aboveground insect parasitoids to antagonistic root-feeding insects change over time, and whether and how plant-odour learning in parasitoids is influenced by interactions that occur in the rhizosphere. I think that this is a novel area of study. Figure 6 summarizes that root-feeding insects influence the foraging efficiency of parasitoids of foliar herbivores via the shared habitat. From a fundamental research perspective, these interactions and the mechanisms that mediate them are another exciting area for future studies.

From an applied perspective, elucidating the factors that influence preference and foraging behaviour of insect parasitoids is important for their application in biological control programs, since insect parasitoids can effectively control pest-populations in agricultural systems. So far, research on refining and improving modern Integrated Pest Management (IPM) programmes has focused on experiments in which above- and belowground systems have been studied independently. The results of this thesis cannot be directly applied to improve biological control strategies, but suggest that to look into the soil might contribute to optimise bio-control programs. What happen in the soil may alter the interactions between the plant shoot, the herbivore pests and their natural enemies. Similarly, elucidating the factors that influence plant preference by leaf-feeding insects is extremely important to improve push-pull strategies in IPM programmes in cropping systems. To date, IPM programmes have been based on research focused on independent above- and belowground systems ignoring the fact that soil dwelling and aboveground insects may strongly interact.

The research carried out in this thesis aimed at disentangling the feed-backs that occur between below- and aboveground insects over several trophic levels. It is likely that horizontal interactions, such as those with other species within the same feeding guild and/or trophic level, will also play a significant role in shaping aboveground-belowground interactions. This has not been addressed in this thesis. The literature is still not very abundant in this area, since most published studies have focused on the effects of single species or functional groups of soil organisms (but see Wurst *et al.* 2004, 2006), on single species of aboveground insects. Considering that soil organisms are likely to interact with each other in many and complex ways, and also considering that it has been recently shown that aboveground plant-insect interactions can dramatically change when a second species enters the scene (Rodriguez-Saona *et al.* 2003, Beckers & Spoel 2006, Zheng *et al.* 2007), inter-species interactions per trophic level will likely affect the outcome of the interactions between above- and belowground food webs.

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Samenvatting

Planten als spin in een web van interacties tussen boven- en ondergrondse insecten
Het is bekend dat planten als vertikale communicatiekanelen kunnen fungeren tussen onder- en bovengrondse plantenetende insecten: worteleters veroorzaken veranderingen in de biomassa en de chemische samenstelling van de bovengrondse delen van een plant, en deze veranderingen kunnen de overleving, de groei en de ontwikkeling van bladeters beïnvloeden. Het belangrijkste doel van mijn promotieproject was te onderzoeken of en hoe de wisselwerking tussen ruimtelijk gescheiden insecten beperkt is tot planteneters (wortel- en spruiteters), dan wel uitgebreid kan worden naar hogere trofische niveaus, zoals bijvoorbeeld parasitaire wespen, de natuurlijke vijanden van de plantenetende insecten. Mijn modelsysteem kende de volgende soorten: zwarte mosterd, *Brassica nigra*, een in het wild voorkomende kruisbloemige (1e trofische niveau), ondergronds de gespecialiseerde worteletende koolvlieg, *Delia radicum* (2e niveau) en de parasitaire wesp *Trybliographa rapae* (3e niveau), en bovengronds het grote koolwitje, *Pieris brassicae*, een gespecialiseerde bladeter (2e niveau), de parasitaire wesp *Cotesia glomerata* (3e niveau) en de hyper-parasitaire wesp *Lysibia nana* (4e niveau). De eerste experimenten lieten zien dat de wortel- en spruitetende insecten elkaar negatief beïnvloeden via de toename aan afweerstoffen in de plant. Deze effecten bleven niet beperkt tot het niveau van de planteneters, maar werkten door op het niveau van de parasitoiden en zelfs op dat van de hyper-parasitoide. Het was opmerkelijk dat de grootste effecten werden gevonden op de parasitaire wespen. Om een voorbeeld te geven: vrouwelijke wespen bleken gastheren op de spruit van planten waarvan de wortels waren aangetast te vermijden, wanneer e rook gastheren beschikbaar waren op planten waarvan de wortels niet waren aangevreten. Waarschijnlijk stellen door de wortelvraat geinduceerde veranderingen in het geurmengsel van de plant de wespen hiertoe in staat. Worteleters bleken zelfs de foerageerbeslissingen van wespen te kunnen beïnvloeden in het geval de bovengrondse gastheren aanwezig waren op onaangetaste planten in de omgeving. Tenslotte werd in een veldexperiment gevonden dat de twee meest voorkomende bladetende insecten bij voorkeur aten van en hun eieren legden op planten waarvan de wortels niet waren aangevreten. Kortom: ook in natuurlijke situaties, waarin op elk moment talloze interacties dooreenlopen, kan het gedrag van spruiteters worden beinvloed door worteleters. De wisselwerking tussen onder- en bovengrondse insecten blijft duidelijk niet beperkt tot de directe belagers van de plant.

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