PLANT-MEDIATED MULTITROPHIC

INTERACTIONS BETWEEN

ABOVEGROUND AND BELOWGROUND

INSECTS

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A mi madre…

Chapter 1

General Introduction

PLANTS, INSECTS AND THEIR INTRICATE INTERACTIONS

Plants and herbivorous insects constitute about one half of the world's macroscopic biodiversity, and represent amongst the most dominant and intricate interactions in terrestrial ecosystems (Strong et al. 1984). Plants have the greatest amount of biomass and insects posses the most species of any class of organisms on earth, whereas half of the currently described insects feed exclusively on plants (Schoonhoven et al. 2005). Since herbivorous insects cannot exist in the absence of plants, and because most species of insect herbivores are relatively specialised feeders (Futuyma and Gould 1979, Chapman 1982, Bernays and Graham 1988), plants and insects have developed mutually antagonistic coevolutionary relationships (Ehrlich and Raven 1964, Breedlove and Ehrlich 1968, Ehrlich and Murphy 1988).

Just as insect herbivores exploit plants, many other arthropods have evolved to attack and consume herbivores. One of the most important groups of natural enemies are the parasitoid wasps (Godfray 1994). Parasitoids are insects whose larvae develop in, or on the bodies of invertebrates, usually other insects, whereas the adults are free-living (Godfray 1994). After parasitism the host invariably dies. Parasitoids have been described in several different insect orders, but are best known from the Hymenoptera (Price 1977, Godfray 1994). Unlike predators, which may need to feed on several preys in order to reach maturity, the resources for parasitoid development are finite and are packed into a single host. Consequently parasitoids are under rigorous selection pressure to optimise use and disposal of these limited host resources (reviewed by Harvey 2005). Parasitoids are considered to be important organisms in terrestrial ecosystems,

not only because of their species richness but also because of their ecological impacts. Parasitoids may contribute to the structure and organisation of communities, by being one of the top-down forces regulating insect herbivore populations (Price et al. 1980, Price et al. 1986, Hassell 1986, Bernays and Graham 1988, Vet and Dicke 1992, Vet 1999). Since the early 1930's parasitoids have been used as model organisms in studies on evolutionary and theoretical ecology (Hassell 1969, Godfray 1994, Hassell 2000). Furthermore, in agricultural systems parasitoids have been long known to play an important role in the biological control of insect pests (Waage 1982, Waage and Hassell 1982, Van Lenteren et al. 1997, Van Lenteren 2000, 2005). Parasitic and predatory insects are attacked by their own predators, and by (hyper) parasitoids and pathogens, which constitute the fourth trophic level. This fourth trophic level may exert a significant negative effect on plant-fitness by removing parasitoids or predators of the herbivores (Rosenheim 1998). However, the impact of these higher trophic level organisms on the structure and functioning of communities has received relatively little attention. In the last three decades, the study of trophic interactions between organisms has evolved from simple plant-herbivore or prey-predator interactions to a more complex approach involving three or more trophic levels simultaneously (plant-herbivore-parasitoid). This multitrophic approach aims to address the complexity of food webs more realistically and to identify the forces that regulate populations of plants, herbivores and carnivores (Price et al. 1980, Turlings et al. 1990, Vet and Dicke 1992).

Currently, there is an increasing interest to link interactions between plants, organisms in the soil and insect communities aboveground. Recent studies have provided evidence that soil dwelling organisms can be a crucial factor influencing interactions between plants and insects in the aboveground domain (Van der Putten et al. 2001, Bardgett and Wardle 2003, Wardle et al. 2004, Bezemer et al. 2005, De Deyn and Van der Putten 2005). The study of the effects of more complex multitrophic interactions between plants and consumers that transcends the soil surface is unquestionably one of the topical themes in

contemporary ecology. It aims to provide a basis for a deeper understanding of the means by which terrestrial communities evolve, assemble and function. The research presented in this thesis aims to contribute to this field by evaluating and discussing interactions between soil and aboveground insects over three to four trophic levels. In order to achieve this objective, the thesis explores the reciprocal effects between belowground and aboveground insect herbivores, and the consequences of these direct plant-mediated interactions on the performance and behaviour of their parasitoids and hyperparasitoids. Furthermore, several processes triggered in the plant have been studied to mechanistically explain the suite of complex above-belowground multitrophic interactions.

PLANT-HERBIVORE INTERACTIONS

For most insect herbivore species, and especially for specialised feeders that constitute over 80% of the herbivorous insects, host plant recognition and acceptance is governed by contact chemoreception of secondary plant metabolites (allelochemicals) (Schoonhoven et al. 2005). Behavioural decisions of plant-feeders to recognise and accept a host plant species, either as a food source or as an oviposition substrate, are predominately based on contact-sensory information (Klijnstra and Schoonhoven 1987, Bernays 1992, Renwick and Chew 1994). Within a species of host plant, individual plant quality is the major guiding force for plant selection by herbivores. Although nitrogen is only one of the many chemical compounds that are of vital importance for insect growth, phytophagous insects in general are limited by nitrogen (White 1984). Therefore, host plants rich in this primary metabolite are generally preferred by insect herbivores.

The preference-performance hypothesis (Jaenike 1978) predicts that there is a strong selection pressure for adults to oviposit on plants with high nutritional quality, involving primary and secondary plant compounds, so that offspring

fitness will be maximised. This hypothesis particularly refers to phytophagous insects with juvenile stages that, following oviposition by the mother, have little or no capacity to choose the host plant on which they feed and grow (this would include larval stages of most Holometabolous insects). In many Hemimetabolous insects (where immature stages are similar to the adult stage) the nymphs can actively select or reject specific food plants. In this case food plant choice may be comparable between adults and larvae, although sensitivity to individual chemicals in the plant may differ (Renwick and Lopez 1999).

Plants have evolved specific physical and chemical defence mechanisms to resist herbivore attack. Physical defences includes the production of morphological structures such as rough cuticles, trichomes, hairs and adhesive glands on the leaf surface, that impede or reduce the colonisation of shoot tissues by herbivores or that reduce the movement and dispersal ability of insects on the plant. Furthermore, most plants produce secondary plant compounds (allelochemicals) that may be toxic and act as feeding deterrents or alter the development and physiology of the feeding herbivores, through reduced rates of growth, smaller size and increased mortality (Schoonhoven et al. 2005). Allelochemicals can also benefit the plants by acting as oviposition and/or feeding deterrents (Karban and Baldwin 1997).

Many insect herbivores are specialised and exclusively feed on plants that produce a particular group of secondary plant compounds (Bernays and Graham 1988). Specialised herbivore species can normally deal with high levels of specific allelochemicals in their diet, via such mechanisms as sequestration or detoxification and excretion, although their development can be negatively influenced when feeding on plant species or genotypes containing excessively high levels of phytotoxins (Adler et al. 1995, Schoonhoven et al. 2005). Allelochemicals, within certain levels, then act as oviposition/feeding stimulants for insects associated to a plant species, and act as deterrents to non-adapted generalist insect herbivores.

Depending on the plant species, allelochemicals can be a constitutively

expressed, increased or chemically modified in response to herbivore damage. Induction of allelochemicals in response to herbivory has been shown in more than 100 plant species (Roda and Baldwin 2003). Low nutritional quality and/or low digestibility of plant tissues is also often regarded as another plant defence strategy against herbivorous insects (Price et al. 1980). However, whether it has evolved to reduce herbivory or is a response to several other selection pressures such as low nutrient availability in soils is controversial and has long been debated (Moran and Hamilton 1980).

PLANT-HERBIVORE-CARNIVORE INTERACTIONS

Natural enemies of herbivorous insects, such as predators and parasitoids, can also be indirectly affected by the host plant through their hosts or preys (Hare 2002). Allelochemicals in the herbivore's diet can exhibit negative effects on the morphology, development, size and survival of carnivores (Harvey 2005, Ode 2006). If the allelochemicals consumed by herbivores are sequestered or diffused through the gut wall and accumulate in tissues such as fat body and hemolymph, the performance of the offspring of natural enemies may be seriously compromised (Campbell and Duffey 1979, Barbosa et al. 1986, 1991, Harvey et al. 2005). In contrast, the growth and development of specialised natural enemies is often less affected by differences in phytotoxins in the food source of the herbivores (Barbosa et al. 1986, Harvey et al. 2005). The nutritional quality of the plant can also influence the growth and development of parasitoids, diminishing (sub-lethal effects) or enhancing the quality of their insect herbivore hosts, and consequently influencing the fitness-related traits of the emerging parasitoids.

Carnivores can also be directly influenced by plants when they are searching for herbivore victims. These effects can be physical, e.g. trichomes that can act as barriers for searching carnivores (Sutterlin and Van Lenteren 1999,

2000). Moreover, carnivore-searching is guided by visual and volatile cues that are produced by herbivores, herbivore-infested plants and/or mutualistic organisms associated with the presence of the herbivore host/prey (Vinson and Iwantsch 1980, Vet et al. 1991, Vet and Dicke 1992, Turlings et al. 1998, Dicke 1999). In response to insect egg deposition (Hilker et al. 2005, Hilker and Meiners 2006) and herbivore attack, plants synthesise and release volatile compounds from damaged and undamaged tissues (Vet and Dicke 1992). These herbivore-induced plant volatiles act as important host and prey-location cues for carnivores (Vet and Dicke 1992, McCall et al. 1993, Dicke 1994, Takabayashi and Dicke 1996, Dicke 1999). Odours coming from the hosts and preys itself are only detected at a much closer proximity than is assumed for plant derived volatiles (Vet and Dicke 1992), and therefore herbivore-induced plant volatiles are of crucial importance for foraging carnivores.

Herbivore-induced plant volatiles can be rather specific in some plant species, differing in response to damage by particular herbivore species, and thus being reliable indicators of the identity of the feeding herbivores for foraging carnivores (De Moreas et al. 1998, Dicke 1999, Vet 1999). However, herbivore-induced plant volatiles may also become far less reliable when the plant is simultaneously attacked by other non-host herbivore species, as often occurs in nature. The volatile composition of plants infested with multiple foliar-feeding herbivore species may differ profoundly from plants infested with a single herbivore species (Shiojiri et al. 2001, 2002). Therefore, multiple-herbivore species infestations may interfere in the foraging behaviour of specialised parasitoids (McCann et al. 1998, McCann 2000, Vos et al. 2001). Interference by non-host herbivores has been theoretically proposed to play an important role in the stabilisation of food webs by generating spatio-temporal refuges (McCann et al. 1998, McCann 2000, Vos et al. 2001, Gols et al. 2005). Under conditions where selection from top-down (natural enemy) pressures is severe, refuges prevent the over-exploitation of herbivore populations allowing for their persistence in space and time (Begon et al. 1996).

Parasitoids are often specialised on one or a few host species and host stages. Therefore, they have evolved complex mechanisms that will allow them to efficiently find their hosts even in challenging environments (Godfray 1994, Dicke 1999, Vet 1999). Choosing a poor quality host in the presence of more suitable hosts can be highly detrimental for the fitness of the developing off-spring of the parasitoid. Therefore, it is expected that natural selection will lead to the evolution of behaviours that increases the capacity of females to preferentially attack hosts of higher quality (Smilowitz and Iwantsch 1975, Iwantsch and Smilowitz 1975, Van Alphen and Vet 1985, Godfray 1994, Brodeur et al. 1998).

Innate responses by foraging parasitoids to volatile plant odours have been shown to change with searching experience. A number of parasitoid species exhibit associative learning of plant odours during host encounters, and are able to adjust their responses to varying host-related cues (Vet and Dicke 1992, Turlings et al. 1993, Vet et al. 1995). This might allow parasitoids to cope with the interference induced by the non-host herbivores feeding on the same plant. How parasitoid behaviour changes depending on experience in complex multitrophic systems is not known.

INFLUENCE OF BELOWGROUND INSECTS ON

PLANT-HERBIVORE INTERACTIONS

In recent years it has been increasingly recognised that soil organisms and ecological processes belowground can significantly influence the structure and function of aboveground systems (Van der Putten et al. 2001, Bardgett and Wardle 2003, Wardle et al. 2004, De Deyn and Van der Putten 2005). Studies exploring interactions between aboveground and belowground biota have commonly focused on the effects of a variety of soil organisms, such as soil decomposers, arbuscular mycorrhizal fungi and root feeders, on aboveground

foliar-feeding herbivorous insects (for a review see Van der Putten et al. 2001, Wardle 2002, Bezemer and Van Dam 2005). In this Chapter-section, I focus on interactions between root feeders, in particular root-feeding insects and plant parasitic nematodes, and foliar-feeding insects that share the same host plant.

So far, no clear pattern emerges that describes the influence of root-feeding insects or plant parasitic nematodes on the performance of foliar-feeding insects sharing the host plant (Van Dam et al. 2003). Effects of root-feeding insects on the performance of their aboveground counterparts have been reported to be neutral (Moran and Whitham 1990, Salt et al. 1996, Hunt-Joshi and Blossey 2005), positive (Gange and Brown 1989, Masters and Brown 1992, Masters 1995, Masters et al. 2001, Poveda et al. 2005) and negative (Birch et al. 1992, Tindall and Stout 2001, Bezemer et al. 2003, Bezemer et al. 2004). Most studies reporting a positive effect pointed to an induced stress response within the plant triggered by the root-feeding insects, which leads to the accumulation of soluble nitrogen, amino acids and carbohydrates in the plant shoot. Such increases in nutrient levels are proposed to stimulate the growth and development of foliar insects (Masters et al. 1993, Bezemer et al. 2002). The mechanisms proposed by a number of studies where negative effects were recorded pointed to increased levels of foliar defense compounds induced by root-feeding insects on the shared host plant (Van Dam et al. 2003, Bezemer et al. 2003, 2004). In a number of plant species, root-feeding by insects has been shown to cause an increase in the level of allelochemicals in the plant shoot, similar to that observed for foliar-feeding chewing insects (Bezemer and Van Dam 2005). It could be argued that specialist herbivores are well adapted to specific allelochemicals in their host plants, and thus foliar-feeding specialists are expected to experience no negative effects of these increased levels phytotoxins caused by root herbivory (Ratzka et al. 2002). However, it remains largely unstudied how and whether changes in the levels of foliar defensive compounds and other metabolites induced by spatially separated herbivores influence the performance of the insects feeding aboveground.

Plant responses to plant-parasitic nematodes are quite variable and their effects on the performance of foliar-feeding insects are difficult to establish. Increases and decreases in levels of secondary plant compounds have been reported on plants exposed to plant-parasitic nematodes, resulting in rather species-specific interactions (Van Dam et al. 2005, Bezemer et al. 2005, Bezemer and Van Dam 2005).

ABOVE AND BELOWGROUND MULTITROPHIC PLANT-INSECT INTERACTIONS

The effects of root feeders on the performance of foliar-feeding insects described as neutral, positive or negative may change if higher trophic levels are taken into account (top-down regulation). Recently, there has been a growing interest in whether and how soil organisms may influence the levels of parasitism of aboveground herbivores (Masters et al. 2001, Gange et al. 2003, Bezemer et al. 2005, Poveda et al. 2005). So far the studies addressing this point report that parasitoids are strongly attracted to plants associated with belowground organisms, independent of the identity of the organisms (e.g. antagonistic root feeders or mutualistic arbuscular mycorrhizal fungi) (Gange et al. 2003, Wurst and Jones 2003, Guerrieri et al. 2004, Poveda et al. 2005). Surprisingly, the influence of soil organisms on induction of plant volatiles aboveground as a possible plant-mediated mechanism to explain, understand and discuss the observed patterns has not been explored. Rasmann et al. (2005) showed that root herbivory induces the release of a volatile compound aboveground, beta-farnesene, from the leaves of the plant. This compound is known to be used by parasitoids of aboveground herbivores to locate their hosts (Turlings et al. 1991). The consequences of such belowground defence induction for the behaviour of aboveground natural enemies of foliar herbivores remain largely unexplored.

Moreover, whether soil organisms can influence the performance of

parasitoids, as mediated through their herbivorous hosts remains largely unstudied. As discussed above, intra- and interspecific variation in plant quality are known to affect parasitoid development via changes in host quality (Campbell and Duffey 1979, Barbosa et al. 1986, 1991, Gunasena et al. 1990, Harvey et al. 2003, Sznajder and Harvey 2003). If root herbivores are able to influence aboveground plant quality affecting the performance of leaf herbivores, these effects might be passed up to higher trophic levels in the food chain. The central objective of this thesis is to explore and mechanistically understand the plant-mediated effects of root-feeding insects in the performance and behaviour of parasitoids of foliar herbivores.

THE MODEL-SYSTEM

In this thesis I explore interactions between spatially separated insects mediated by the host plant. I use a naturally occurring system in the Paleartic realm (Fig. 1). The model-system consists of the wild cruciferous plant species *Brassica nigra* L. (Brassicaceae) (first trophic level), the specialist root herbivore species *Delia radicum* L. (Diptera: Anthomyiidae) (second trophic level, belowground), its natural enemy, the parasitoid *Trybliographa rapae* W. (Hymenoptera: Figitidae) (third trophic level, belowground), the specialist foliar-feeding herbivore species *Pieris brassicae* L. (Lepidoptera: Pieridae) (second trophic level, aboveground), its natural enemy the parasitoid *Cotesia glomerata* L. (Hymenoptera: Braconidae) (third trophic level, aboveground), and the secondary parasitoid (hyperparasitoid) *Lysibia nana* G. (Hymenoptera: Ichneumonidae) (fourth trophic level, aboveground) (Fig. 1).
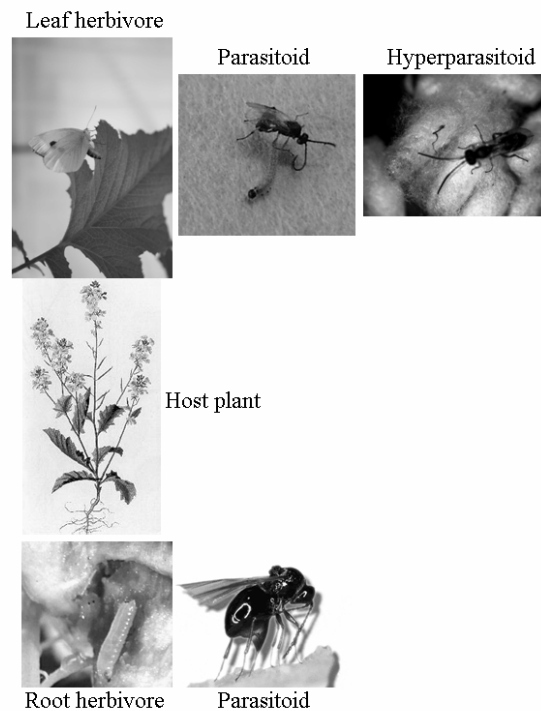
Figure 1. Representation of the model-system

Brassica nigra

Brassica nigra, commonly know as black mustard, is a widely distributed summer-annual plant in the family Brassicaceae. This plant generally grows in extended patches along rivers and disturbed areas, sometimes being the dominant species in early successional habitats (Schaminée et al. 1998). Cruciferous plants posses potent inducible direct and indirect defences via the production of glucosinolates and their breakdown products, providing excellent potential for the study of multitrophic interactions.

Glucosinolates are a class of about 100 naturally occurring thioglucosides, which are secondary plant compounds that are characteristic of the Brassicaceae and related families in the order Capparales (Kjaer 1977, Schoonhoven et al. 2005). Glucosinolates appear to have little biological impact themselves, but they can be converted into biologically active products such as isothiocynates (mustard oils), nitrites and thiocyanates upon enzymatic degradation by myrosinase enzymes. Hydrolysis of glucosinolates occurs immediately after plant tissue is ruptured and probably also takes place during normal catabolism but at

much lower rates (Kjaer 1981).

Glucosinolates are present in all parts of the plant and concentrations differ according to plant species, tissue type, physiological age, plant health and nutrition. Glucosinolates and their breakdown products are toxic and unpalatable to many generalist feeders that do not normally feed on plants containing those compounds. Glucosinolates are known to be strong feeding- and oviposition-stimulants to many specialist insects that have crucifers as host plants (Renwick and Lopez 1999). However, at higher dosages they can act as deterrents or have detrimental effects on the growth, development and survival of several crucifer-feeding insects (Agrawal and Kurashige 2003, Müller et al. 2003, Van Dam and Raaijmakers 2006). The cell damage that facilitates the hydrolysis of glucosinolates also produces a range of volatile catabolites that include nitriles and isothiocynates. Isothiocynates are thought to provide parasitoids with cues that can be utilised to locate their hosts from a distance (Reed et al. 1995).

The major glucosinolate in *B. nigra* roots and shoots is sinigrin (2-propenyl or allyl glucosinolate). Sinigrin makes up more than 99% of the glucosinolates found in the plant shoots (Van Dam et al. 2005). Sinigrin, which is an aliphatic glucosinolate, and indole glucosinolates, (especially isothiocynates formed upon leaf damage) can reduce feeding damage by both generalist and specialist herbivores (Agrawal and Kurashige 2003, Van Dam et al. 2005).

Delia radicum and *Trybliographa rapae*

Delia radicum (Diptera: Anthomyiidae) is a specialist chewing root herbivore that feeds on several species of crucifers. Females lay eggs on the soil surface around the plant stem, and the newly hatched maggots feed by boring galleries in the upper roots of the plant, pupating in the soil at the end of the juvenile development. The feeding activity of larvae on the plant roots cause rapid infection by microorganisms. *D. radicum* is associated with gut-microbial symbionts, which contribute to host nutrition directly as a food source as well as by providing increased digestive potential to digest refractory plant materials

(Lukwinski et al. 2006). Furthermore, the infection caused by the associated gut-microrganisms, the damage caused in the roots as a consequence of larval feeding provides a point of entry for subsequent infection by endemic root-rot pathogens (Soroka et al. 2004). The symbiotic bacteria as well as the rot root-associated microorganisms may also influence the direct and indirect plant defence system. There is evidence that sulphide compounds can be emitted by bacterial metabolism of root exudates and decomposition of plant material, and are diffused aboveground (Romeis et al. 2003). Little is known however, about interactions between root herbivores and their associated microorganisms, and the role that this may play in aboveground interactions. It is important to note that, for the model system used in this thesis the effects of root herbivory cannot be fully separated from the effects of the microorganisms associated to *D. radicum*.

Trybliographa rapae (Hymenoptera: Figitidae) is one of the main parasitoids of *D. radicum* in Europe (Fournet et al. 2004). This solitary koinobiont endoparasitoid parasitises first to third instar larvae of *D. radicum*, as well as a few other closely related species. Female parasitoids forage for hosts via antennation, ovipositor probing and vibrotaxis. Females use their sharp, but flexible ovipositor to explore infested roots to find hosts and to evaluate their quality (Neveu et al. 2000). The wasps readily parasitise larvae that are actively feeding in the root-galleries, but refuse hosts extracted from the root.

Pieris brassicae, *Cotesia glomerata* and *Lysibia nana*

Pieris brassicae (Lepidoptera: Pieridae), the large cabbage white butterfly, is a specialist chewing foliar herbivore that feeds on several species of wild and cultivated plants in the Capparales. The female butterfly typically lays broods of 7-100 eggs on the underside of a leaf. After hatching, the larvae feed gregariously until the third instar, when they disperse on the food plant. The larvae of pierid butterflies, including *P. brassicae*, feed exclusively on plants that contain glucosinolates. The glucosinolate sinigrin is also reported to act as

feeding stimulant for *P. brassicae* and *P. rapae* larvae (Renwick and Chew 1994, Renwick and Lopez 1999).

Sequestration of plant toxins in herbivore-hemolymph of foliar-feeding insects could potentially be used by the phytophagous insects for their own defence against natural enemies (Duffey and Scudder 1972). There has been some debate as to whether *P. brassicae* actively sequesters glucosinolates. Aplin et al. (1975), for instance, found evidence for sequestration, but a much more recent study by Müller et al. (2003) found no evidence of sequestration in *P. brassicae* larvae. It was originally proposed that *P. brassicae* larvae, which exhibit contrasting yellow and black colouration, were advertising toxic repellence. Recent work, however, has shown that the larvae preferentially feed from flowers of *B. nigra* and related plants, which are also bright yellow and that the colouration actually provide camouflage for the larvae (J.J.A. Van Loon, personal communication).

The genus *Cotesia* belongs to the family Braconidae, subfamily Microgastrinae (Hymenoptera), and is one of the largest genera of parasitoid wasps. A number of species of this genus are employed frequently as biological control agents against phytophagous insects. *Cotesia* sp. are also used as model organisms in studying the physiology, ecology and population genetics in parasitoids and other insects. This genus has considerable importance from both applied and basic scientific perspectives. *Cotesia glomerata* (Hymenoptera: Braconidae) is a gregarious endoparasitoid that commonly parasitises first to third instar larvae of few genera of the Pieridae, with *P. brassicae* being its preferred host in Europe (Levin et al. 1983). Adults are ready to mate immediately after emergence and the fertilised females start seeking for hosts. The fecundity of the females varies between 1000 to 2000 eggs, and females lay approximately 20 to 30 eggs per larval host (Levin et al. 1983). After parasitism, the larval host continues feeding and growing until it reaches its final (L5) instar (Karowe and Schoonhoven 1992). Hosts parasitised by *C. glomerata* and other gregarious microgastrines exhibit variable patterns of growth that is brood size

dependent, with hosts with large parasitoid loads typically attaining much higher terminal weights than hosts with light parasitoid loads (Harvey 2000). This pattern contrasts with solitary species that usually reduce the growth of their hosts (Strand et al. 1988, Harvey and Thompson 1995, Harvey et al. 1999, 2004a). These association-specific differences in growth and development of parasitised hosts is frequently mediated by polydnaviruses, venoms, and other parasitoid-derived products that originate from the ovipositing female or her progeny (Vinson and Iwantsch 1980, Beckage and Gelman 2004). *Cotesia glomerata* females can distinguish between healthy and previously parasitised hosts after insertion of their ovipositor, although, like many solitary and gregarious microgastrines, this parasitoid readily superparasitises (Vandriesche 1988, Gu et al. 2003).

Lysibia nana (Hymenoptera: Ichneumonidae) is a solitary secondary (hyperparasitoid) ectoparasitoid that parasitises fully cocooned pre-pupae and pupae of several closely related gregarious primary endoparasitoids in the subfamily Microgastrinae, with *C. glomerata* being one of its preferred hosts (Harvey et al. 2003, 2004b, 2006). Adult females perforate the host-cocoon and inject permanent paralyzing venom into the host when ovipositing (Harvey et al. 2007). Like many other ectoparasitic idiobionts, adult body size is strongly correlated with host size at parasitism (Harvey et al. 2006, 2007). *L. nana* exhibits a remarkably efficiency utilizing host tissues attaining a similar body mass as *C. glomerata* adults (98%) that developed in cocoons of a comparable size (Harvey et al. 2006).

RESEARCH OBJECTIVES AND OUTLINE OF THE THESIS

Soil organisms, whose presence often pass unnoticed, can play a major role structuring aboveground plant-insect communities by affecting the survival, growth and development of foliar-feeding insects (reviewed by Van der Putten et

al. 2001). The general aim of this thesis is to study the consequences of these spatially separated interactions on the performance and behaviour of higher trophic levels, and to understand the mechanisms that mediate the interactions between such distant organisms.

In Chapter 2, I evaluate the influence of belowground (root-feeding) insects on the performance of a four trophic-level food chain aboveground, composed by the plant, a leaf herbivore, a parasitoid and a hyperparasitoid species, when the root- and foliar-feeding insects share a host plant. In Chapter 3, I evaluate the influence of aboveground (foliar-feeding) insects on the performance of a belowground food chain composed by the plant, a root herbivore and a parasitoid, when sharing the same host plant. Chapters 2 and 3 describe how the root and shoot associated insects influence each others' growth, development, and survival, and whether the effects influence higher trophic levels in the food chains affecting the assemble of the community of insects. In both chapters, changes in plant quality and biomass were assessed to link with the observed effects. The results of Chapters 2 and 3 reveal that root and foliar-feeding insects negatively influence each other performances, as well as the performance of the higher trophic levels in the food chain. I show that the effects are correlated with increased levels of secondary plant compounds (allelochemicals) within the shared host plant.

The results described in Chapters 2 and 3 raise the question of whether the suboptimal performances achieved by the root and shoot herbivores and their higher trophic levels as a result of sharing a host plant, will have consequences for the functioning of the community. In Chapter 4 I address this question by studying whether adult female parasitoids prefer to search for and parasitise hosts feeding on root-damaged or root-undamaged plants, keeping in mind that the parasitoid's off-spring develop significantly poorer on hosts feeding on root-damaged plants. I also assess differences in the composition of the plant volatile blend emitted following root and foliar herbivory to correlate with the behavioural responses observed. The results show that parasitoids prefer to

parasitise hosts feeding on root-undamaged plants, in a choice situation with plants exposed to root herbivory and root-undamaged plants. This behaviour leads to optimal food conditions for their off-spring. I show that the mechanism mediating these interactions points to changes in the volatile blend of the shared plant, induced by the root-feeding insects.

Subsequently, in Chapter 5 I examine whether the effects of root-feeding insects on the behaviour of aboveground parasitoids can be mediated by other plants in the shared habitat. I describe an experiment designed to investigate whether root-feeding insects influence the searching efficiency of adult *C. glomerata* females when the aboveground and belowground herbivores do not share the host plants. In a large tent-experiment, I manipulated the proportion of *B. nigra* plants that were exposed to root herbivory and recorded the time that *C. glomerata* females took to locate other plants in the same habitat on which larvae of the host *P. brassicae* were present. I show that parasitoid searching efficiency increases when there are plants present in the shared habitat that are exposed to root herbivory. These results suggest that spatially separated insects can interact over several trophic levels even when the belowground and aboveground herbivores are not connected by a plant, but by the mere coexistence in the same habitat.

Field observations are of crucial importance to validate patterns and conclusions generated from studies under strictly controlled conditions in the laboratory. In Chapter 6, in the field I evaluate the influence of belowground insects on host plant selection of naturally occurring foliar-feeding insects of *B. nigra* plants, as an indirect measurement of the impact that belowground root-feeding insects exert on the functioning of aboveground trophic chains in nature, where several other complex interactions take place. I show that some species of naturally occurring leaf insect herbivores of *B. nigra* preferentially colonise and feed on root-undamaged plants over root-damaged plants. Remarkably, in the field the effects of root herbivory also depend on the level of aggregation of plants with and without root herbivory.

A general discussion of the results is presented in chapter 7. Here, I also discuss future directions to develop in the topical theme of linking and understanding interactions between spatially separated organisms.

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Chapter 2

Root herbivore effects on aboveground herbivore, parasitoid and hyperparasitoid performance via changes in plant quality

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SUMMARY

1. Plants and insects are part of a complex multitrophic environment, in which they closely interact. However, most of the studies have been focused mainly on bi-tritrophic aboveground subsystems, hindering our understanding of the processes that affect multitrophic interactions in a more realistic framework.
2. We studied whether root herbivory by larvae of the fly *Delia radicum* can influence the development of the leaf feeder *Pieris brassicae,* its parasitoid *Cotesia glomerata* and its hyperparasitoid *Lysibia nana,* through changes in primary and secondary plant compounds.

3. In the presence of root herbivory, the development time of the leaf herbivore and the parasitoid significantly increased, and the adult size of the parasitoid and the hyperparasitoid were significantly reduced. The effects were stronger at low root fly densities than at high densities.

4. Higher glucosinolate (sinigrin) levels were recorded in plants exposed to belowground herbivory, suggesting that the reduced performance of the aboveground insects was via reduced plant quality. Sinigrin contents were highest in plants exposed to low root fly densities, intermediate in plants exposed to high root fly densities and lowest in plants that were not exposed to root herbivory.

5. Our results show, for the first time, that root herbivory via changes in plant quality can reduce the performance of an aboveground multitrophic level food chain. This underlines the importance of integrating a broader range of aboveground and belowground organisms to facilitate a better understanding of complex multitrophic interactions and inter-relationships.

INTRODUCTION

Most plants employ a range of direct defense strategies to protect them from attacking herbivores. These include the production of morphological structures such as trichomes on the leaf surface, which may impede herbivore colonization or movement, and/or the production of toxic compounds (allelochemicals) that can act as feeding deterrents or alter the development and physiology of herbivores, through reduced rates of growth, smaller size and increased mortality. However, the performance of natural enemies of herbivores may be seriously compromised by toxins consumed by herbivores and sequestered in their tissues. A number of studies have indeed reported that allelochemicals contained in host or prey diet negatively affect the development and survival of their parasitoids or predators (reviewed by Hare 2002).

Studies exploring interactions over several trophic levels have traditionally focused on the aboveground community (e.g. Harvey et al. 2003). However, it is becoming increasingly clear that plants and insects are part of a complex multitrophic aboveground and belowground environment, in which they closely interact (Van der Putten et al. 2001). Therefore, by focusing exclusively on the aboveground subsystem, our understanding of the processes that affect multitrophic interactions in a more realistic framework has been hindered. Over the past two decades attention has been paid to interactions between aboveground and belowground insect herbivores sharing the same host plant. It is now acknowledged that herbivorous insects can indirectly interact even when they are spatially or temporally separated from other herbivores associated with the same host plant (Gange and Brown 1989, Masters 1995, Masters and Brown 1997, Gange 2001, Masters et al. 2001).

Thus far, most above- belowground interaction research has been limited to two trophic levels (Moran and Whitham 1990, Masters and Brown 1992, Salt et al. 1996). However, a recent study reported that densities of aboveground parasitoids may be increased by root feeders (Masters et al. 2001). Studies with other soil organisms, such as earthworms and arbuscular mycorrhizal fungi, have also shown that there is the potential for effects of soil organisms on the third trophic level aboveground (Gange et al. 2003, Wurst and Jones 2003). In the field, parasitoids may also be attacked by hyperparasitoids and predators, which may play an important role by influencing community structure (Brodeur 2000). However, we are not aware of any study that has examined the effects of root herbivores on the fourth aboveground trophic level. Changes in plant quality have been shown to affect hyperparasitoid fitness, via herbivore and parasitoid effects (Harvey et al. 2003). Thus, the effects of root herbivores may trickle up to the fourth trophic level aboveground.

Interactions between above- and belowground insect herbivores and their antagonists may be mediated by primary (nutrients) and secondary (phytotoxins) plant chemistry. Two hypotheses have been proposed to explain above-

belowground herbivore interactions with opposite predictions for the effects of root feeders on aboveground herbivores (reviewed by Bezemer et al. 2002). The 'stress response hypothesis' predicts that root herbivores induce a stress response in the host plant that reduces water and nutrient uptake and leads to the translocation and accumulation of soluble nitrogen and carbohydrates in aboveground plant parts, ultimately enhancing the performance of the foliar feeders (Masters et al. 1993). The 'defense induction hypothesis', argues that root herbivory induces a defense response in the plant that can lead to the accumulation of secondary plant compounds aboveground, which in turn can reduce the performance of the foliar feeders (Bezemer et al. 2003, 2004a, Van Dam et al. 2003). Studies of the effects of root-feeding insects on aboveground herbivores have provided evidence for both hypotheses and it appears that both mechanisms are not mutually exclusive. However, as far as we know, no study has thus far examined if changes in primary and/ or secondary plant compounds induced by belowground herbivory can also influence the performance of parasitoids, predators and even higher trophic levels.

This study examines the effects of root herbivory over four trophic levels of an aboveground system, as possibly mediated by changes in primary and secondary plant compounds. Using the wild cruciferous plant *Brassica nigra* L. (Brassicaceae), we studied the effects of root herbivory by *Delia radicum* L. (Diptera:Anthomyidae) larvae on an aboveground system consisting of the herbivore *Pieris Brassicae* L. (Lepidoptera: Pieridae), the parasitoid *Cotesia glomerata* L. (Hymenoptera:Braconidae) and its secondary hyperparasitoid, *Lysibia nana* Gravenhorst (Hymenoptera:Ichneumonidae).

We selected a cruciferous plant species because wild crucifers possess potent inducible direct defenses via the production of glucosinolates and their breakdown products, and thus provide great potential for the study of multitrophic interactions (Van Dam et al. 2003). *Brassica nigra* is particularly interesting because glucosinolate production is at least three to five times higher than in related cultivated species, such as *Brassica oleracea* L. (Harvey et al.

2003). Furthermore, the expression of secondary plant compounds in aboveground shoots of *B. nigra* is known to be influenced by root herbivory (Van Dam et al. 2004). *Pieris brassicae* is a specialist chewing herbivore that only feeds on plants containing glucosinolates. However, despite this specialization, a negative correlation between larval feeding and glucosinolate (sinigrin) levels has been observed in several *Pieris* species (Olsson and Jonasson 1994, Traw and Dawson 2002a). *C. glomerata* is a fairly specialised gregarious koinobiont endoparasitoid that parasitizes first to third instar larvae of *P. brassicae* and related species. *Lysibia nana* is a solitary idiobiont hyperparasitoid that parasitizes pre-pupae and pupae of *Cotesia glomerata* and related species in the Microgastrinae.

We specifically addressed the following questions: (1) Is the growth, development and survival of aboveground herbivores, parasitoids and hyperparasitoids affected by belowground herbivory? (2) Are the effects of root herbivory consistent across the aboveground trophic spectrum (e.g. are the effects on development time and body size [positive or negative] the same in all of the organisms under investigation? (3) If variation in the performance of aboveground insects is observed in response to the presence or absence of root herbivory, can these results possibly be attributed to changes in the primary and secondary plant compounds of aboveground shoots as affected by belowground herbivory (thus testing the 'stress-response' and 'defense induction' hypotheses)? Lastly, we discuss the importance of linking above- and belowground multitrophic interactions with processes occurring in larger ecological communities.

MATERIALS AND METHODS

Pieris brassicae (leaf herbivore) and *Cotesia glomerata* (parasitoid) were obtained from an insect culture maintained at the Laboratory of Entomology of

Wageningen University, The Netherlands. *P. brassicae* and *C. glomerata* were cultured on *Brassica oleracea*. *Lysibia nana* (hyperparasitoid) was obtained from a culture maintained at the Netherlands Institute of Ecology, Heteren, The Netherlands. The hyperparasitoid was cultured exclusively on pre-pupae and pupae of *C. glomerata*. *Delia radicum* (root herbivore) was obtained from an insect culture of the Swiss Federal Research Station for Fruit-Growing, Wädenswil, Switzerland. Root flies were cultured on the roots of *Brassica napus* and *B. oleracea*.

 Brassica nigra seeds were collected from a single *B. nigra* population in the Northwest of Wageningen, The Netherlands. Seeds were surface sterilised and germinated on glass beads. One week after germination seedlings were transplanted into 1.2 litre pots filled with soil collected from a restoration area that was abandoned in 1996, at De Mossel in Ede, The Netherlands. The sandy loam soil had been sieved (2 cm) mixed with 25% white sand, and sterilised using gamma radiation (25 KGray) to eliminate all soil organisms. The plants were grown in a greenhouse, at a temperature of $25 \pm 1^{o}C$ (day) and $22 \pm 1^{o}C$ (night), with a relative humidity of 70% and with a photoperiod of 16:8 hours (day:night). Natural daylight was supplemented by 400W metal halide bulbs (one per 1.5 m^2). Plants were watered daily and were supplemented with nutrients once when the plants were three weeks old.

 Forty five days after transplanting, *D. radicum* larvae were introduced into the experimental pots. Depending upon treatment, 5 (low density) or 20 (high density) newly hatched first instar larvae were carefully placed with a small brush next to the main stem of the plant and observed to ensure that they successfully crawled into the soil. A third set of plants was kept undamaged and served as control. Ten plants were used for each root herbivore density (0, 5, and 20).

 It is known that the leaf glucosinolate concentration in *Brassica* plants increases after some days of feeding by *P. brassicae*. For *B. nigra* it takes approximately seven days to reach the maximum glucosinolate concentration

(N.M. Van Dam, personal communication). To clearly visualize the potential effect of the root herbivore on the aboveground insects, it is necessary to discern it from the effects that *P. brassicae* could cause. A second set of replicates was set up with this purpose. In this extra set of plants, *P. brassicae* larvae were periodically moved to a new host plant (every three to four days throughout the course of the experiment). For this, a second series of plants was transplanted and inoculated with *D. radicum* at different days, so that the time between inoculation and exposure to aboveground herbivory was kept constant.

Aboveground insect measurements

Thirteen days after root fly inoculation, plants were placed individually into meshed cylindrical cages (height 1 m, diameter 35 cm). Into each cage two unparasitised and two parasitised second instar larvae of *P. brassicae* were introduced by placing them on the eighth mature leaf of the plant. Parasitism was carried out by individually offering second instars of *P. brassicae* to single *C. glomerata* females in plastic vials.

Development of unparasitised *P. brassicae* larvae was checked daily and fresh pupae were weighed on a Mettler Toledo Microbalance (accuracy ± 1 µg). Larval development time of *P. brassicae* was determined as the number of days until pupation. To measure the amount of plant tissues consumed by early larval instars of *P. brassicae,* all feeding damage on each leaf was marked on a transparent sheet and the total area per plant eaten was measured using a leaf-area scanner. The measurements were performed on the plant renewal set of plants, after the first (consumption during days one to four) and second (consumption during days five to seven) plant renewal.

For parasitised *P. brassicae,* the number of emerged cocoons per clutch was counted. Larval development time was determined as the number of days between parasitism and prepupal egression; pupal development time was determined as number of days between prepupal egression and adult emergence. From each clutch, five cocoons were randomly selected for hyperparasitism. The

remaining *C. glomerata* cocoons of each clutch were placed, per a given clutch, into a Petri dish (10 cm diameter), and checked daily (at intervals of 2 hours) for adult emergence. At emergence, adult size and development time were recorded. Each cocoon presented to *L. nana* was individually weighed on the microbalance. Cocoons were presented in groups of five with three to five *L. nana* females in Petri-dishes (diameter 10 cm). Upon parasitism, cocoons were immediately removed and transferred individually into plastic vials (2 cm diameter, 5 cm length). Cocoons were checked daily, and at eclosion adult hyperparasitoids were weighed on the microbalance. Egg to adult development time was also recorded.

Plant responses

Foliar glucosinolate concentrations (GLS), nitrogen (N), phosphorus (P), potassium (K) and carbon/nitrogen ratio (C:N) were measured in an additional set of plants exposed to low and high levels of root herbivory and in undamaged control plants. Plants were treated as described above and the period of root herbivory was also the same.

To determine GLS, for each plant two immature (the second and third leaf) and two mature leaves (the ninth and tenth) were removed from the plant using a razor blade and immediately frozen at -80 $^\circ$C, then freeze-dried and ground. Foliar GLS were also measured after the plants were exposed to root herbivory and aboveground herbivory (17 days of root herbivory with during the last four days also aboveground herbivory). GLS were determined using High Performance Liquid Chromatography (HPLC) as described by Van Dam et al. (2004).

To determine the content of foliar N, P and K and C:N ratio the remaining leaves of each plant were harvested, oven-dried at 70 $^\circ$C, ground and analysed as described in Troelstra et al. (2001). Total foliar and root biomass were also measured.

Statistical analysis

The statistical analysis of the performance of *P. brassicae*, *C. glomerata* and *L. nana* was based on a model with two factors (root herbivory and plant renewal). Since more than one observation was taken from each experimental unit (pot-plant) there are two sources of experimental error, that of sub-samples (within each experimental unit) and between experimental units. Within a mixed model framework these two random terms can be specified. The following mixed model describes the experimental set up:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + d_{ij} + e_{ijk}$$

where Y_{ijk} is the observed value, μ is the general mean, α_i the fixed effect of the plant renewal level i (i=with or without renewal), β_j (the fixed effect of root fly density; j=0, 5, 20 larvae per pot), and $\alpha\beta_{ij}$ the plant renewal by density interaction. The two random terms in the model correspond to the between pot variation d_{ij} (σ^2_d) and the within pot variation e_{ijk} (σ^2_e), both assumed independently and normally distributed with variances δ^2_d and δ^2_e respectively. Model fit was done by employing restricted maximum likelihood (REML) in Genstat 6 and statistical tests for fixed effects were done using an approximated F-test. Normality, independence and homogeneity of variance were checked by inspection of the residuals after fitting the model.

The effect of root herbivory and aboveground herbivory on glucosinolates and primary plant compound levels in plant tissues were analysed using a fixed model:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \lambda_k + \alpha\beta_{ij} + \alpha\lambda_{ik} + \beta\lambda_{jk} + \alpha\beta\lambda_{ijk} + e_{ijkl}$$

where Y_{ijkl} is the observed value, μ is the general mean, α_i corresponds to the effect of above ground herbivore i (i= with or without renewal), β_j the effect of root fly density (j=0, 5, 20 larvae per pot), λ_k the effect of leaf age i (i=1,2; young

and old respectively). Finally, all two-way and the three-way interaction between the factors were included ($\alpha\beta_{ij}$, $\alpha\lambda_{ik}$, $\beta\lambda_{jk}$, $\alpha\beta\lambda_{ijk}$). Residuals where assumed independently and normally distributed with variance δ^2_e. Analyses were carried out using STATISTICA 7. Normality, independence and homogeneity of variance were checked by inspection of the residuals after model fit.

RESULTS

Herbivore performance

Root herbivory significantly affected larval development time of *P. brassicae,* and the effect differed between root herbivore densities (Table 1; Fig. 1a). Relative to control plants, larval development time was extended by, on average, 0.7 days at low root herbivore densities, but no effect was observed at high densities. Pupal weight was not affected by root herbivory (Table 1; Fig. 1b). Early instar food consumption also did not differ between treatments, neither during the first period (day one to four, $F_{2,12} = 0.41$; $P = 0.66$) nor the second (day 5 to 7, $F_{2,12} = 0.80$; $P = 0.47$; data not shown). There was no effect of plant renewal on *P. brassicae* performance (Table 1).

Table 1. Approximate F-test for the fixed effects and variance component estimates from REML analysis of the effect of plant renewal and root herbivory on the larval development time and pupal weight of the herbivore *Pieris brassicae* and on the larval development time, cocoon weight, and clutch size of the parasitoid *Cotesia glomerata.*

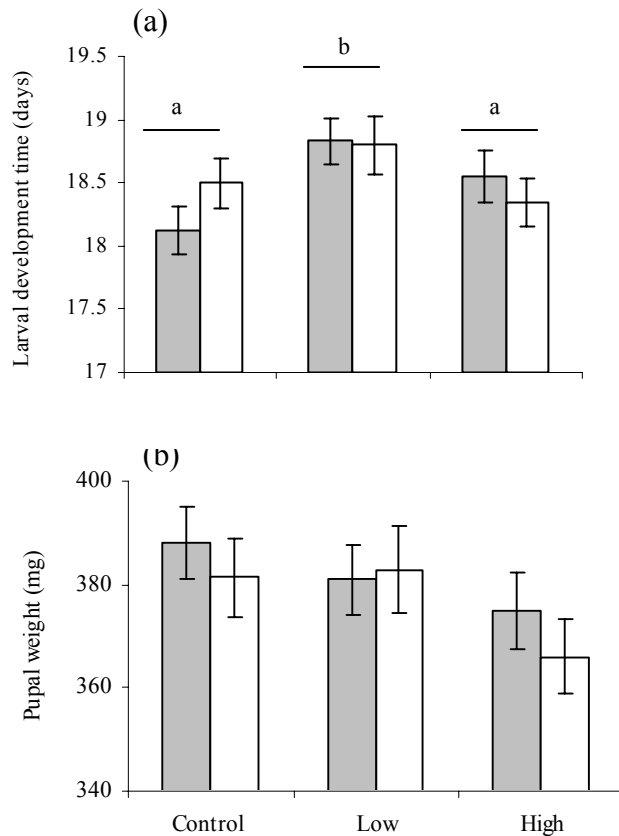| | | Herbivore | | | | Parasitoid | | | | | |
| | | Larval development | | Pupal weight | | Larval development | | Cocoon weight | | Clutch size | |
Factors	d.f.	F	P	F	P	F	P	F	P	F	P
Plant renewal	1	0.01	0.93	0.83	0.36	0.01	0.91	0.42	0.51	0.11	0.73
Root herbivory	2	3.67	0.02	2.12	0.12	9.10	< 0.001	6.23	0.002	4.86	0.008
Interaction	2	1.17	0.30	0.30	0.74	2.33	0.09	0.99	0.37	1.69	0.18

(a)

(b)

Figure 1. Mean larval development time (a) and pupal weight (b) of *Pieris brassicae* on *Brassica nigra* plants that were exposed to low or high root herbivore densities, or without root herbivory (Control). No plant renewal = shaded bars; plant renewal = open bars. Although there were no significant plant renewal effects, the data is shown for the renewal and no renewal treatment. Significant differences (P < 0.05) between root fly density treatments are indicated by different letters, based on a Tukey test.

Parasitoid performance

There were highly significant effects of root herbivory on *C. glomerata* larval development time, cocoon weight, and clutch size, and the effects differed between root herbivore densities (Table 1; Fig. 2a-c). Larval development time was significantly longer on plants exposed to the low root herbivore densities compared to undamaged plants and plants exposed to the high densities of root herbivores (Fig. 2a). Independent of root herbivore density, parasitoid cocoon weight was significantly reduced in the presence of root herbivores (Fig. 2b). Secondary parasitoid clutch size was significantly higher on plants exposed to low root herbivore densities compared to undamaged plants or plants exposed to high root herbivore densities (Fig. 2c). There was no significant effect of plant renewal on parasitoid larval development time, cocoon weight or clutch size (Table 1).
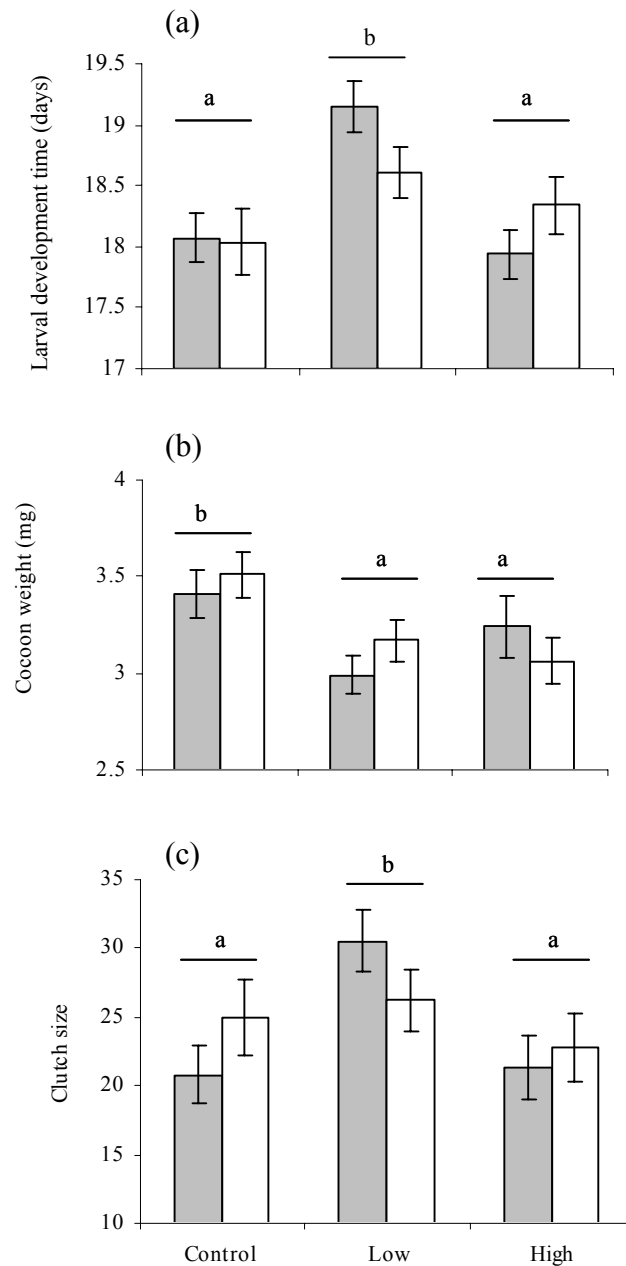
Figure 2. Mean larval development time (a), cocoon weight (b) and clutch size (c) of *Cotesia glomerata* on *Brassica nigra* plants that were exposed to low or high root herbivore densities or without root herbivory (Control). No plant renewal = shaded bars; plant renewal = open bars. Although there were no significant plant renewal effects, data shows the renewal and no renewal treatment separately. Significant differences ($P < 0.05$) between root fly density treatments are indicated by different letters, based on a Tukey test.

Both parasitoid pupal development time and parasitoid adult weight were affected differently by parasitoid sex, plant renewal, and root herbivory, resulting in a significant three-way interaction for both variables (Table 2). There was a strong effect of sex on pupal development time, with males developing faster than females (Table 2). Without plant renewal, female pupal development time was not affected by root herbivory, but with plant renewal, females had shorter development times on plants exposed to low root fly densities (Fig. 3a). Male adult parasitoid weight was not affected by root herbivory or plant renewal, but in the treatment without plant renewal female weight in plants exposed to low and high root fly densities was lower than in control plants (Fig. 3b). Mortality was not significantly affected by root herbivory ($F_{2,81} = 0.42$; P = 0.66; data not shown).

Table 2. Approximate F-test for the fixed effects and variance component estimates from REML analysis of the effect of sex, plant renewal and root herbivory on the pupal development time and adult weight of the parasitoid *Cotesia glomerata*.

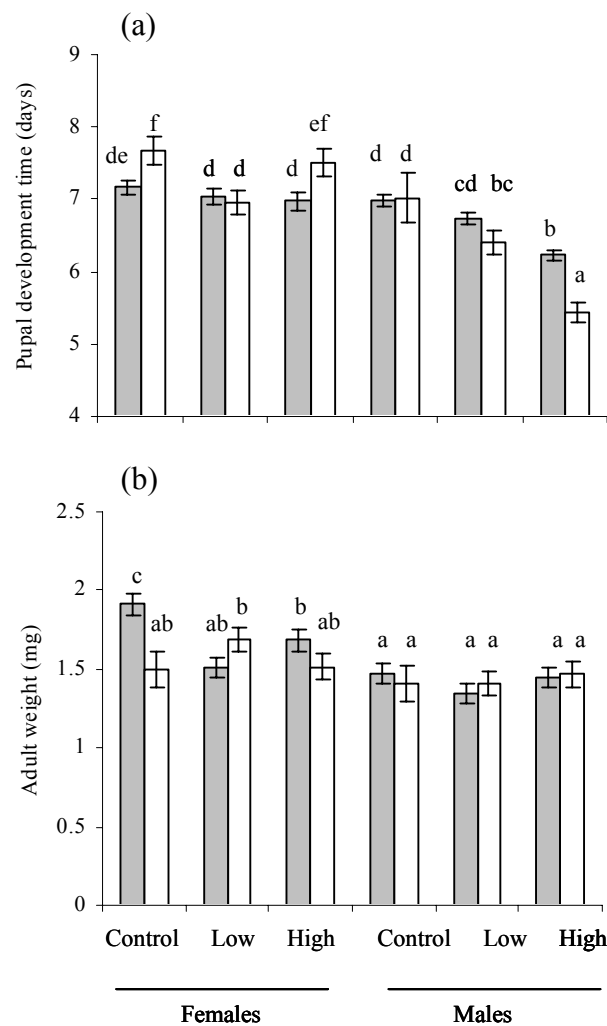| Factors	d.f.	Pupal development		Adult weight	
		F	P	F	P
Sex (1)	1	94.73	< 0.001	230.26	< 0.001
Plant renewal (2)	1	7.94	0.005	0.44	0.50
Root herbivory (3)	2	30.71	< 0.001	1.23	0.29
Interaction (1.2)	1	29.02	< 0.001	8.48	0.004
Interaction (1.3)	2	10.72	< 0.001	11.01	< 0.001
Interaction (2.3)	2	1.92	0.146	2.57	0.07
Interaction (1.2.3)	2	4.24	0.01	10.07	< 0.001

(a)

(b)

Figure 3. Mean pupal development time (a) and adult weight (b) of *Cotesia glomerata* on *Brassica nigra* plants that were exposed to low or high root herbivore densities or without root herbivory (Control). No plant renewal = shaded bars; plant renewal = open bars. Bars with identical letters are not significantly different (P < 0.05), based on a Tukey test.

Hyperparasitoid performance

Independent of sex, *L. nana* adult weight was significantly affected by root herbivory and plant renewal (Table 3), while egg to adult development time was not affected (Table 3; Fig. 4a). In the no renewal treatment, in the presence of root herbivores, hyperparasitoid weight was significantly reduced compared to the control treatment (Fig. 4b). Adult hyperparasitoid weight was lower for adults emerging from the renewal treatment than from the no renewal treatment.

Mortality was not significantly affected by root herbivory ($F_{2,55} = 1.01$; $P = 0.36$; data not shown).

There was a strong positive relationship between hyperparasitoid adult weight and parasitoid cocoon weight (host size) ($F_{1,201} = 176.1$; $P < 0.001$) but this relationship was not affected by root herbivory treatment ($F_{2,201} = 0.61$; $P = 0.54$) (Fig. 5).

Table 3. Approximate F-test for the fixed effects and variance component estimates from REML analysis of the effect of sex, plant renewal and root herbivory on the pupal development time, and adult weight of the hyperparasitoid *Lysibia nana.*

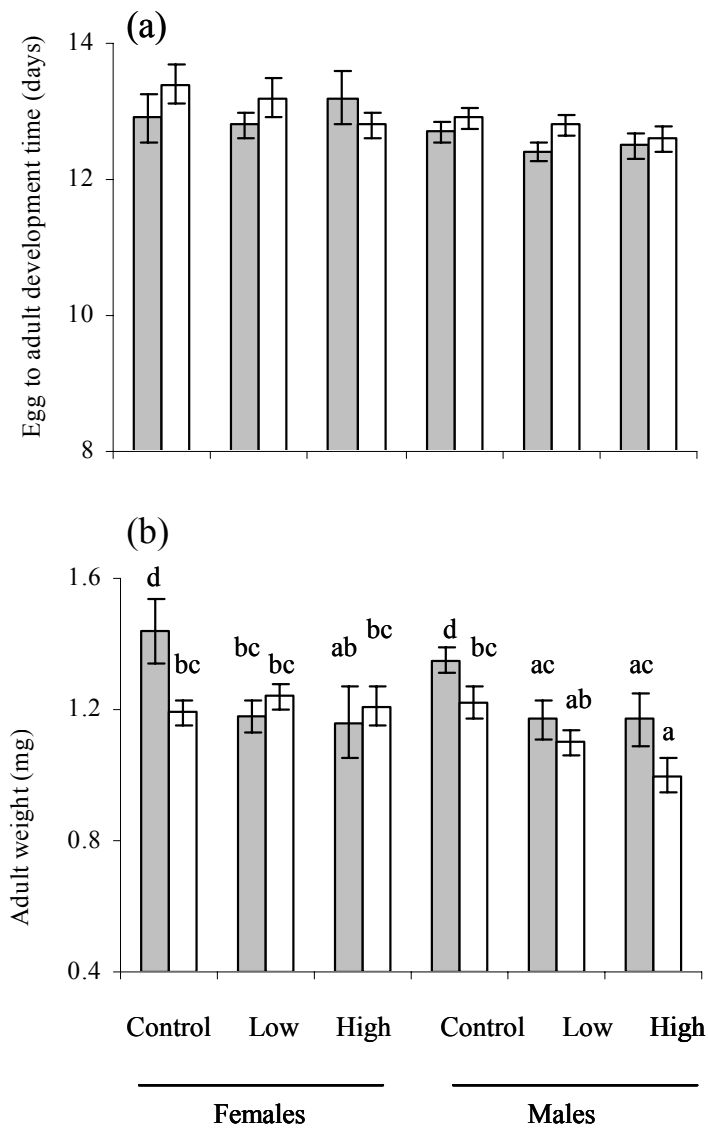| Factors	d.f.	Pupal development		Adult weight	
		F	P	F	P
Sex (1)	1	7.38	0.007	2.70	0.10
Plant renewal (2)	1	3.04	0.081	6.21	0.013
Root herbivory (3)	2	1.42	0.24	6.93	< 0.001
Interaction (1.2)	1	0.26	0.60	2.53	0.11
Interaction (1.3)	2	0.25	0.78	1.06	0.34
Interaction (2.3)	2	0.74	0.47	0.72	0.48
Interaction (1.2.3)	2	0.95	0.38	1.87	0.15

Figure. 4. Egg to adult development time (a) and adult weight (b) of *Lysibia nana*, on *Brassica nigra* plants that were exposed to low or high root herbivore density or without root herbivory (Control). No plant renewal = shaded bars; plant renewal = open bars. Bars with identical letters are not significantly different (P < 0.05), based on a Tukey test.
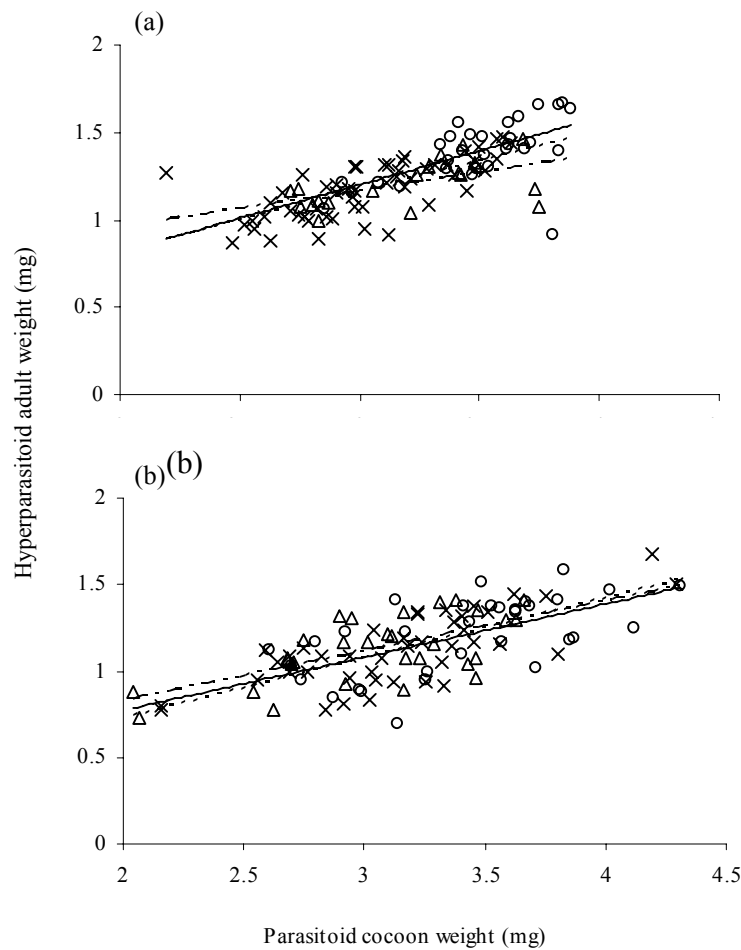
Figure 5. Relationship between *Cotesia glomerata* cocoon fresh weight and emerging *Lysibia nana* weight, developed on *Brassica nigra* plants that were exposed to low (crosses, short dotted line) or high (triangles, long dotted line) root herbivore densities or control plants without root herbivory (circles, solid line), on the no plant renewal (a) and plant renewal (b) set of plants. Regression equations from control plants (y_0), low root fly densities (y_5) and high root fly densities (y_{20}): ($r^2 = 46$): $y_0 = 0.078 + 0.35x$; $y_5 = 0.14 + 0.32x$; $y_{20} = 0.31 + 0.27x$.

Plant responses

Plants in all treatments had similar shoot biomass ($F_{2,21} = 0.22$; $P = 0.80$), but root herbivory significantly decreased root biomass ($F_{2,21} = 4.76$; $P = 0.01$). Plants exposed to high root herbivory had significantly lower root biomass compared to undamaged plants, while intermediate root biomass was observed in plants exposed to low root herbivore density (Table 4). Foliar nitrogen ($F_{2,24} = 4.88$; $P = 0.03$) and C:N ratio ($F_{2,22} = 8.93$; $P = 0.005$) were significantly affected by root herbivory. Nitrogen levels were significantly lower in plants exposed to high root

herbivore densities compared with control plants and plants exposed to low root herbivory , resulting in significantly higher carbon/nitrogen ratios in plants with high root herbivory (Table 4). No change in P and K content of mustard foliage was recorded.

Table 4. Chemical composition (mean ± SE) in shoot tissue and root and shoot biomass of *Brassica nigra* plants exposed to low or high root herbivore densities and in undamaged control plants. Root herbivory was caused by *Delia radicum*. Within rows, means followed by identical letters are not significantly different (P < 0.05) based on a Tukey test.

	Control	Low	High
% Nitrogen	3.79 ± 0.26 b	3.79 ± 0.37 b	2.77 ± 0.04 a
C:N ratio	10.35 ± 0.27 a	11.19 ± 0.96 a	14.19 ± 0.17 b
% Phosphorous	0.57 ±0.06	0.60 ± 0.04	0.69 ± 0.12
% Potassium	4.04 ± 0.27	4.33 ± 0.35	4.37 ± 0.33
Root biomass (g)	1.11 ± 0.09 a	0.93 ± 0.10 ab	0.61 ± 0.10 b
Shoot biomass (g)	2.73 ± 0.20	2.61 ± 0.10	2.55 ± 0.10

Foliar glucosinolate content was significantly increased in presence of root herbivory (Table 5). The changes in total glucosinolate levels were mainly caused by changes in the major glucosinolate, sinigrin, which represented 99.7 % of the total glucosinolate contents. In presence of root herbivory sinigrin content significantly increased in young leaves (Fig. 6a) but not in mature leaves (Fig. 6b). Sinigrin levels were significantly higher at low root herbivore densities than in undamaged plants while intermediate level of sinigrin was observed in plants exposed to high root herbivore density (Fig. 6a). There were also strong effects on sinigrin levels following four days of aboveground herbivory (Table 5). In young leaves, sinigrin levels were significantly lower in presence of aboveground herbivory (Fig. 6a).

Table 5. ANOVA of the effect of aboveground herbivory, root herbivory and leaf age on sinigrin levels of *Brassica nigra* plants. Root herbivory was caused by *Delia radicum* larvae and aboveground (AG) herbivory was caused by *Pieris brassicae* larvae.

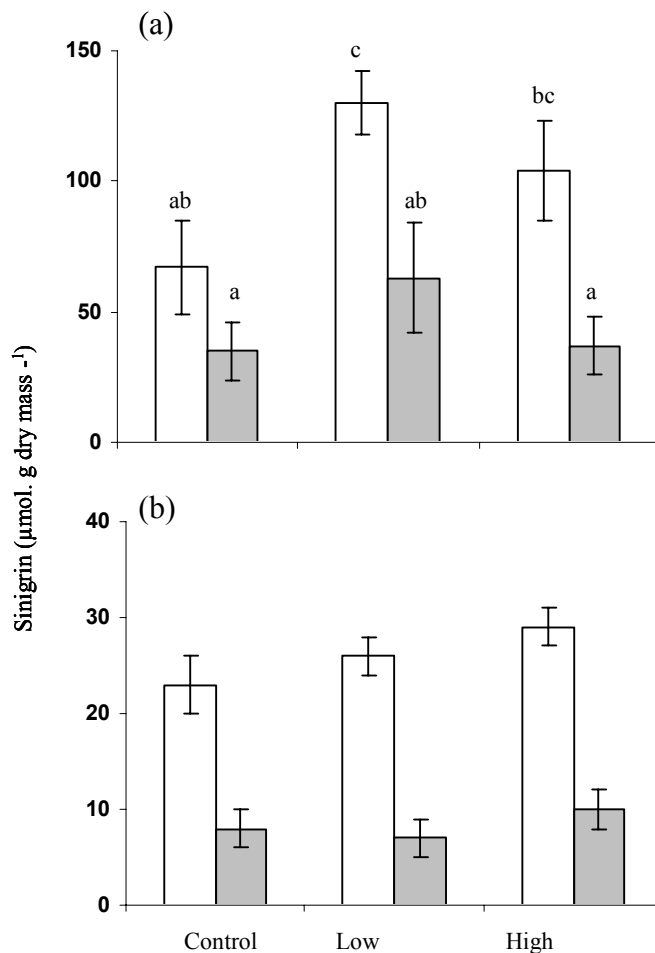| Factors	d.f.	F	P
AG herbivory (1)	1	28.86	< 0.001
Root herbivory (2)	2	3.97	0.02
Leaf age (3)	1	67.76	< 0.001
Interaction (1.2)	2	0.97	0.38
Interaction (1.3)	1	7.69	0.007
Interaction (2.3)	2	3.83	0.02
Interaction (1.2.3)	2	0.61	0.54
Error	48		

Figure 6. Sinigrin levels in young (a) and mature leaves (b) of *Brassica nigra* plants exposed to low or high root herbivore densities or without root herbivory (Control), without (open bars) and with (shaded bars) four days of aboveground herbivory. Root herbivory was caused by *Delia radicum* larvae and shoot herbivory was caused by *Pieris brassicae* larvae. Bars with identical letters are not significantly different (P < 0.05), based on a Tukey test.

DISCUSSION

This experiment is the first to provide evidence that root-feeding insects, via the shared host plant, can influence not only aboveground herbivores and parasitoids, but also hyperparasitoids. Our study also reveals that effects of root herbivory do not uniformly influence the performance of all aboveground insects, but that they can mostly bypass one trophic level while negatively affecting others higher up in the aboveground food chain.

Larval development times of *P. brassicae* and *C. glomerata* were extended in both cases by about one day on plants exposed to root herbivores. The developmental programme of many endoparasitoids is known to be physiologically coordinated with biochemical characteristics of the host, including endocrinological factors such as juvenile and prothoracic hormone titres (Beckage 1985, Lawrence 1990). Many koinobiont parasitoids, including *C. glomerata*, do not begin destructive feeding and thus commence exponential larval growth, until the host enters its final instar (Harvey et al. 1994). This ensures that sufficient resources are available for parasitoid progeny to complete their development. Consequently, the increase in development time observed in *C. glomerata* is therefore most probably attributable to a concomitant increase in the development time of its herbivore host*, P. brassicae*. The increase in development time that was observed in *P. brassicae* and *C. glomerata* associated with plants exposed to *D. radicum* was not, however, carried over to *L. nana.* Idiobiont parasitoids, such as *L. nana*, attack hosts which do not feed or grow, and thus which represent mostly 'static' resources (Mackauer and Sequeira 1993).

Different effects were observed for the response on pupal or adult weight. While pupal weight of *P. brassicae* was not significantly affected, cocoon weight of *C. glomerata* and adult weight of *L. nana* were significantly reduced in the presence of root herbivory. This suggests the transmission of a qualitative effect from the plant via the herbivore, to the parasitoid and the hyperparasitoid. Adult

body size in idiobionts is often strongly correlated to the initial size of the host on which they developed (Mackauer and Sequeira 1993), and this is also true for *L. nana* (Harvey et al. 2003). Thus, the reduction in body size of the hyperparasitoid when developing on *C. glomerata* cocoons originating from plants exposed to *D. radicum* is probably attributable to the negative quantitative effects of root herbivory on the size of the primary parasitoid host.

Sinigrin content increased by about 50 percent in plants exposed to low root fly densities compared with control plants, whereas in plants exposed to high root fly densities levels were intermediate. Other work has shown that root herbivory by *Delia floralis* can almost double the amount of glucosinolates in shoots of cultivated *Brassica* species (Birch et al. 1992). Sinigrin is the primary glucosinolate produced by *B. nigra* plants, and is known to act as a feeding deterrent for many herbivorous insect pests of *Brassica* and *Sinapis* species (Olsson et al. 1994, Schoonhoven and Liner 1994). Our results suggest that the reduced performance of aboveground insects on plants previously exposed to root herbivory may have been the indirect consequence of allelochemicals in the host plant, induced by *D. radicum* belowground. Our results thus at least partially support the 'defense-induction hypothesis'.

The amount of root biomass was greatly reduced when plants were exposed to high root fly densities. However, the maximum concentration of secondary plant defense compounds (sinigrin) were recorded on plants exposed to low root fly densities. It is possible that plants exposed to high root fly densities were severely stressed and thus unable to maintain optimal direct defense capabilities, as suggested by a decrease in the N content of their foliage. On the other hand, plants exposed to low root fly densities, which experienced less root damage, retained their vigour and were thus able to allocate defense compounds to the shoots more effectively.

Specialist herbivores of crucifers are thought to have evolved ways to minimize the toxic effects of the glucosinolates (Ratzka et al. 2002). Glucosinolates can stimulate feeding of specialist herbivores (Bodnaryk 1991),

but other reports have shown no effect (Bodnaryk and Palaniswamy 1990) or a negative effect on the feeding and/or development of specialists (Stowe 1998, Agrawal and Kurashige 2003). Our results indicate that although *P. brassicae* is a specialist herbivore and is thought to be well adapted to high levels of glucosinolates (e.g. Harvey et al. 2003), indirect interactions with belowground herbivores can change plant quality (via the increased production of defense compounds to shoots) enough to impact their development and that of its parasitoid and hyperparasitoid.

The plant renewal factor was added with the aim of excluding the possible effects of foliar-feeding on the expression of aboveground secondary compounds. In that context, it is important to note that we did not find a significant effect of plant renewal on *P. brassicae* and *C. glomerata* development. Moreover, the interaction between renewal and other factors were mostly insignificant with the sole exceptions of the parasitoid pupal development time and adult weight that showed a less straightforward and more complex three-way interaction. The only main effect of renewal was observed on the adult weight of the hyperparasitoid, where wasps were larger in the no-renewal treatment than in the renewal treatment. This suggests that the performance of most aboveground insects was negatively affected irrespective of the renewal treatment under investigation. Several factors may explain why differences were not observed in these experiments. First, it is possible that *P. brassicae* did not cause additional induction of glucosinolates in the plants. Alternatively, the effects of root herbivory may have been much stronger than aboveground herbivory.

Foliar nitrogen levels were also negatively affected by root herbivory, but only in plants exposed to the highest densities of *D. radicum*. It is well established that nitrogen is a limiting factor for insect growth. Increasing the levels of nitrogen in plants that initially contained deficiencies in this nutrient has been shown to enhance the growth of many organisms, including insects (Mattson 1980). However, this is unlikely to have been a major factor affecting

the development of the aboveground insects in this study, because nitrogen levels were very high to begin with, irrespective of treatment, and because insect performance was most negatively affected when associated with plants exposed to low root fly densities that had nitrogen levels similar to controls.

In summary, this is the first study to have shown that root herbivores, such as *D. radicum*, can exert indirect influences over the development of at least three trophic levels of an aboveground system. These affects are driven, in part, by differences in plant quality that are probably the result of changes in levels of secondary plant compounds as influenced by damage in the roots of the host plant. Most importantly, these results have significant implications for our understanding of community-level processes in the aboveground and belowground domains. They emphasize the need to acknowledge the role of both aboveground and belowground biota in determining the structure and function of ecological communities (Bezemer et al. 2004b, Wardle et al. 2004). By integrating a broader range of aboveground and belowground systems, future work will help to facilitate a better understanding of complex multitrophic interactions.

Acknowledgements

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Chapter 3

Impact of foliar herbivory on the development of a root-feeding insect and its parasitoid

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SUMMARY

The majority of studies exploring interactions between aboveground and belowground biota have been focused on the effects of root-associated organisms on foliar herbivorous insects. This study examines the effects of foliar herbivory by *Pieris brassicae* L. (Lepidoptera: Pieridae) on the performance of the root herbivore *Delia radicum* L. (Diptera: Anthomyiidae) and its parasitoid *Trybliographa rapae* (Westwood) (Hymenoptera: Figitidae), mediated through a shared host plant *Brassica nigra* L. (Brassicaceaea). In presence of foliar herbivory the survival of *D. radicum* and *T. rapae* decreased significantly by more than 50 %. In addition, newly emerged adults of both root herbivores and

parasitoids were significantly smaller on plants that were exposed to foliar herbivory compared to control plants. To determine what factor(s) may have accounted for the observed results, we examined the effects of foliar herbivory on root quantity and quality. No significant differences in root biomass were found between plants with and without shoot herbivore damage. Moreover, concentrations of nitrogen in root tissues were also unaffected by shoot damage by *P. brassicae* larvae. However, higher levels of indole glucosinolates were measured in roots of plants exposed to foliar herbivory, suggesting that the development of the root herbivore and its parasitoid may be, at least partly, negatively affected by increased levels of these allelochemicals in root tissues. Our results show that foliar herbivores can affect the development not only of root-feeding insects but also their natural enemies. We argue that such indirect interactions between above and belowground biota may play an important role in the structure and functioning of communities.

INTRODUCTION

Over the past twenty years, studies exploring community-level processes have been primarily based on bi- and tritrophic interactions in aboveground systems, usually involving associations between plants, insect herbivores, and their natural enemies such as parasitoids or predators (e.g. Price et al. 1980, Turlings et al. 1990, Vet and Dicke 1992, Dicke 1999). While providing valuable data on a range of selective forces shaping community structure and function, these studies have generally ignored biological processes that occur belowground. Recently, however, it has become apparent that aboveground trophic interactions can be strongly influenced by interactions between the host plant and soil dwelling organisms (Gange and Brown 1989, Masters and Brown 1992, 1997, Masters et al. 1993, Masters 1995, Gange 2001, Van der Putten et al. 2001, Bardgett and Wardle 2003, Bezemer et al. 2003). For instance, recent studies have shown that

soil dwelling organisms, such as root-feeding insects, arbuscular mycorrhizal fungi and nematodes can also influence the growth, development and behaviour of higher trophic levels aboveground, including parasitoids (Masters et al. 2001, Gange et al. 2003, Wurst and Jones 2003, Bezemer et al. 2005) and even hyperparasitoids in the fourth trophic level (Soler et al. 2005).

Alternatively, it is also known that aboveground herbivory can affect the development of belowground insect herbivores (reviewed by Blossey and Hunt-Joshi 2003), when the shared host plant is previously exposed to intense shoot damage. For example, Tindall and Stout (2001) reported that severe damage by insect foliar herbivores was correlated with a significant reduction in the body size of an insect herbivore feeding on root tissues of the same host plant, compared with conspecifics developing on roots of plants without foliar herbivores. Other studies have shown similar effects (Masters et al. 1993, Hunt-Joshi and Blossey 2005). However, the effects of foliar herbivory on the development of natural enemies in the soil, such as predators and parasitoids, remain largely unexplored.

Thus far the effects of aboveground herbivory on the performance of belowground consumers were mainly explained by quantitative changes in the amount of root biomass caused by aboveground herbivory, when extreme amounts of leaf damage result in partial or complete death of the root system. However, minor levels of shoot damage, while not leading to a significant reduction in root biomass, may still affect the quality of the root tissues influencing the development of root-associated organisms (reviewed by Bezemer and Van Dam 2005). Furthermore, interactions between above and belowground herbivores may also be mediated by qualitative changes in the shared host plant. For example, insect herbivory frequently leads to a systemic defense response within the host plant, resulting in enhanced concentrations of secondary plant compounds in plant organs that have not been damaged by herbivory (Karban and Baldwin 1997). Secondary plant compounds commonly have a negative impact on herbivore performance (Karban and Baldwin 1997). Several studies

have shown that root herbivory can result in enhanced concentrations of secondary plant compounds in the foliage, although the reverse, e.g. the effects of foliar herbivory on changes in concentrations of root secondary plant compounds, have rarely been reported (reviewed by Bezemer and Van Dam 2005). Moreover, herbivory can induce a stress response within the host plant, which can lead to a reallocation of plant compounds such as carbohydrates and soluble nitrogen between root and shoot tissues, and the concentration of these compounds is known to directly affect insect growth parameters (Masters et al. 1993, Masters and Brown 1997). It is important to stress that the mechanisms described above are not necessarily mutually exclusive.

In this study we examine the effects of aboveground herbivory on the development and survival of a belowground insect herbivore and its endoparasitoid. Our study is based on interactions involving a naturally occurring system in Western Europe. The black mustard, *Brassica nigra* L. (Brassicaceae), is a widely distributed annual crucifer that is common along rivers and on disturbed sites in The Netherlands (Schaminée et al. 1998). It is attacked by several adapted specialist herbivores, including the large cabbage white butterfly, *Pieris brassicae* L. (Lepidoptera: Pieridae) whose larvae feed on the shoots and flowers of this and related species (Harvey et al. 2003). Mustard plants are also attacked by specialised root-feeding herbivores, including the cabbage root fly, *Delia radicum* L. (Diptera: Anthomyiidae) (Coaker and Finch 1971). *Trybliographa rapae* (Westwood) (Hymenoptera: Figitidae) is one of the main parasitoids of *D. radicum* in Europe (Lahmar 1982). The main aim of this investigation is to determine if the growth, development and survival of *D. radicum* and its endoparasitoid, *T. rapae*, are affected by foliar herbivory by *P. brassicae*. We also examine if aboveground herbivory leads to qualitative and/or quantitative differences in root tissues. We compare levels of root biomass in plants with or without shoot damage, as well as levels of nitrogen in root tissues. Finally, we compare levels of allelochemicals (glucosinolates) in root tissues of plants in the two treatments. We hypothesize that aboveground herbivory by *P.*

brassicae will result in reduced root-plant quality and this will negatively affect the performance of *D. radicum*. Because *T. rapae* is a koinobiont parasitoid, *D. radicum* larvae continue feeding and growing during much of the parasitoid-host interaction (reviewed by Harvey 2005). As a consequence of this close association between the host and the parasitoid we hypothesize that the effects of *P. brassicae* on *D. radicum* will be carried over to *T. rapae*. Since parasitoids are highly susceptible to very small changes in the quality of the hosts, we hypothesize that the effect on the parasitoid will be stronger than in the host.

MATERIALS AND METHODS

Studied species

Brassica nigra, like other members of the Brassicaceae, possesses potent inducible direct defenses via the production of glucosinolates and their breakdown products, and thus the plant provides excellent potential for the study of multitrophic interactions (Van Dam et al. 2003). *Pieris brassicae* is a specialist chewing foliar herbivore that feeds on several species of wild and cultivated crucifers. The female butterfly typically lays broods of 7-140 eggs on the underside of a leaf. After hatching, the larvae feed gregariously until the third instar, when they disperse on the food plant. *Delia radicum* is a specialist chewing root herbivore that feeds on several species of crucifers. Females oviposit near the root-stem interface. The newly hatched maggots feed by boring galleries in the upper roots of the plant. *Trybliographa rapae* is a fairly specialised solitary koinobiont endoparasitoid that parasitizes L1-L3 instar larvae of *D. radicum,* as well as several closely related species. Adult parasitoid females forage via antennation, ovipositor probing and vibrotaxis in order to find their hosts (Vet and Van Alphen 1985). The wasps readily parasitize larvae that are actively feeding in the root-galleries, but refuse hosts extracted from the root (Jones 1986). Females use their sharp, but flexible ovipositor to explore infested

roots to find hosts and to evaluate their quality (Neveu et al. 2000).

Seeds of *B. nigra* were collected from a single population in the Northwest of Wageningen, The Netherlands. Seeds were surface sterilised and germinated on a bed of glass-pearls (pearls of 1 mm diameter). One week after germination, seedlings were transplanted into 1.2 litre pots, filled with soil collected from a restoration area that was abandoned in 1996, at De Mossel in Ede, The Netherlands. The sandy loam soil was sieved (2 cm) and mixed with 10% white sand (to improve the drainage of the soil), and sterilised using gamma radiation (25 KGray) to eliminate all soil organisms. The plants were grown in a greenhouse, at 22 ± 1^oC (day) and 16 ± 1^oC (night), 70% relative humidity and 16:8 hours day:night. Natural daylight was provided by metal halide lamps (225 $\mu mol\ s^{-1}\ m^{-2}$ PAR; 1 lamp per 1.5 m^2). Plants were watered daily and supplemented with nutrients (Hoagland solution) once when the plants were three weeks old. *Pieris brassicae* was obtained from the insect culture maintained at the Laboratory of Entomology of Wageningen University, The Netherlands. *D. radicum* and *T. rapae* were obtained from insect cultures maintained at the Laboratory of Ecobiology of Insect Parasitoids, of Rennes University, France.

Experimental design and insect measurements

To test the effect of aboveground herbivores on the performance of soil insects, we reared *D. radicum* and its parasitoid *T. rapae* on roots of plants previously exposed (treatment) and not exposed (control) to *P. brassicae* larvae. Twenty five days after transplanting, three newly-hatched L1 larvae of *P. brassicae* were carefully placed with a small brush on the youngest fully developed leaf of each plant, and enclosed in a cylindrical clip cage (5.5 cm diameter) in order to ensure that the damage was localised. After 3 days, the larvae were moved to another new leaf on the plant. The larvae were allowed to feed for three more days, and then each larva and clip cage was removed. Thus, each foliar-damaged plant was exposed to feeding by three L1 *P. brassicae* larvae for a total of six days. A

second set of plants was kept undamaged and served as a control. To standardize the possible effects of the clip cages on plant shoots, clip cages were also placed on control plants, following the same methods as for the foliar-damaged plants. A total of seventy-two plants were used in the experiment (36 plants with foliar herbivory and 36 without foliar herbivore damage).

One day after removing the *P. brassicae* larvae, four L1 *D. radicum* larvae were introduced to each plant by carefully placing the larvae with a brush onto the soil surface immediately adjacent to the stem. Thirty minutes after inoculation, each plant was carefully checked with a magnifying glass to ensure that all the root fly larvae had successfully entered into the soil. For each plant, two of the larvae were healthy and two had been parasitised by *T. rapae* approximately within the past 12 h. In a previous study, we reported that there was no significant difference in final root biomass in *B. nigra* plants with initial densities of five unparasitised *D. radicum* larvae per plant compared with undamaged plants (Soler at al. 2005). Furthermore, parasitised larvae consume less plant material and attain smaller terminal masses than healthy hosts (Grandgirard 2003), thus we assumed that plant root biomass would not be a limiting factor for root herbivore/parasitoid development. It is also important to stress that larvae of *D. radicum* are not cannibalistic (A.M. Cortesero unpublished observations), making it possible for unparasitised and parasitised larvae to share a host plant.

To obtain parasitised *D. radicum* larvae, approximately 300 first instar larvae were offered to 60 mated *T. rapae* females over the course of four hours. To facilitate and ensure high rates of parasitism, a slice of *B. napus* (turnip) root of 1.5 to 2 cm of height (15 cm diameter) that was previously infested with the larvae of *D. radicum* was offered to the parasitoid females. The larvae were collected from the root after four hours and 144 larvae were then transferred to the experimental *B. nigra* plants. Because direct observations of parasitism are almost impossible in *T. rapae*, 100 additional larvae from the infested turnip slice were removed and placed on a new turnip, where parasitoid emergence was

monitored. From these 100 larvae 92 *T. rapae* adults emerged, whereas none of the larvae produced *D. radicum* adults.

Following the belowground inoculation of unparasitised and parasitised *D. radicum* larvae, experimental plants were placed individually into large meshed cylindrical cages (height 1 m, diameter 35 cm), to ensure that newly emerged adult flies and wasps did not escape and could be immediately captured. Plants from the two treatments were randomly distributed in the greenhouse, and were moved around the room every 2-3 days to avoid any potential effects of positioning on insect development. The cages were checked twice daily (once in the morning and once in the afternoon) to record the emergence of adult flies and parasitoids. Newly emerged adults of *D. radicum* and *T. rapae* were collected with an aspirator. At emergence, the following data was recorded: development time between inoculation and adult eclosion in days, and adult body size, based on the measurement of hind tibia length in both the herbivore and parasitoid. Hind tibia length has been commonly used as a measure of adult size in many insects, especially parasitoids (e.g. Godfray 1994, Harvey et al. 1994, Bezemer and Mills 2003). The hind tibia length of *D. radicum* and *T. rapae* were measured using a calibrated slide under a stereomicroscope. Adult survival (calculated as the proportion of insects that emerged relative to the number of larvae introduced in each pot) was also recorded.

Plant responses

Because the measurement of glucosinolate and nitrogen concentrations requires the destructive sampling of plants, root samples were taken from an additional set of plants (9 plants per treatment). The plants in the additional set were treated exactly as the foliar-damaged and control plants described above. Root material was collected 1 h before the other set of experimental plants was inoculated with parasitised and unparasitised *D. radicum* larvae to evaluate the quality of the control and foliar-damaged plants that larvae encountered after inoculation. After carefully washing the roots of each plant, the fine roots were separated from the

main root. Roots less than 1 mm thick were considered to be fine roots (with a hair-like appearance) and roots thicker than 1 mm were considered to be main roots. The roots were immediately frozen at -80 $^{\circ}$C, then freeze-dried and ground. Glucosinolate contents were determined using High Performance Liquid Chromatography (HPLC) as described by Van Dam et al. (2004). Nitrogen content was determined using a Carlo Erba/Fisons, type EA1108, CHN analyzer. The total root biomass of the experimental plants was determined at the end of the experiment. The roots of each plant were harvested, oven-dried at 70 $^{\circ}$C and subsequently weighed on a microbalance.

Statistical analysis

The effect of foliar herbivory (with or without foliar herbivory) and larval status (parasitised or unparasitised) on survival of *D. radicum* and *T. rapae* was tested by a Generalised Linear Mixed Model (with binomial distribution and logit link function, including plant as random term). The effect of foliar herbivory on developmental time and hind tibia length of *D. radicum* and *T. rapae* was tested by a Generalised Linear Model (with normal distribution and identity link function). The effect of foliar herbivory and root type (primary or secondary roots) on plant quality (nitrogen and glucosinolate levels) was tested using a Linear Mixed Model, including plant as random term to consider that the fine and main roots were sampled from the same plants. To test the effect of foliar herbivory on plant quantity (root biomass) we used Analysis of Variance. Normality, independence and homogeneity of variance were checked by inspection of the residuals after model fitting. Analyses were carried out in Statistica and Genstat.

RESULTS

Foliar herbivory significantly affected the survival of the root herbivore *D. radicum* and its parasitoid *T. rapae* (Wald = 12.17; P <0.001). Relative to control plants, the survival of *D. radicum* and *T. rapae* decreased by more than 50 %, when the host plants were previously exposed to foliar herbivory (Fig. 1), and the effect was qualitatively similar for root herbivore and parasitoid responses indicated by a non-significant interaction term (Wald = 0.49; P = 0.48). There was a significant effect of foliar herbivory on *D. radicum* and *T. rapae* adult size, measured as hind tibia length (Wald = 5.88; P = 0.02). Herbivore and parasitoid adult-size were both reduced in the presence of foliar herbivores on the shared host plant (Fig. 2). Again, the negative effect of foliar herbivory did not differ between host and parasitoid (interaction term Wald = 0.02; P = 0.86). Development time of *D. radicum* and *T. rapae* was not significantly affected by foliar herbivory (Wald = 3.34; P = 0.07).
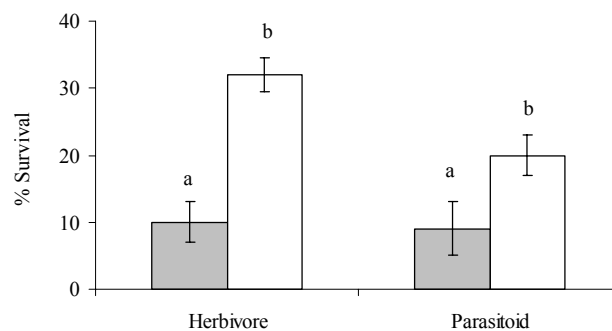

Figure 1. Mean (± SE) survival of *D. radicum* (herbivore) and *T. rapae* (parasitoid) on *B. nigra* plants that were exposed to foliar herbivory (by *P. brassicae*) (shaded bars), and on control plants (open bars). Within herbivore and parasitoid, significant differences (P < 0.05) between treatments are indicated by different letters.

Figure 2. Mean (± SE) hind tibia length of *D. radicum* (herbivore) and *T. rapae* (parasitoid) on *B. nigra* plants that were exposed to foliar herbivory (by *P. brassicae*) (shaded bars), and on control plants (open bars). Within herbivore and parasitoid, significant differences ($P < 0.05$) between treatments are indicated by different letters.
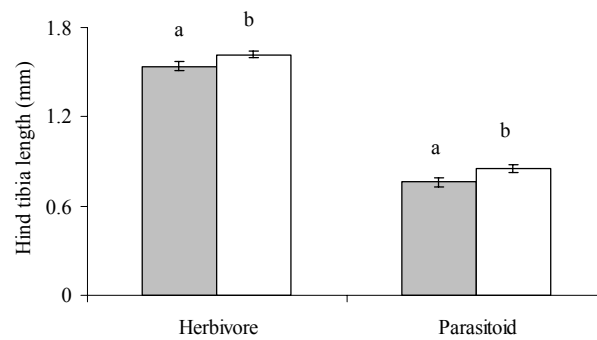
Plants in both treatments had similar root biomass ($F_{1,30} = 0.008$; $P = 0.92$) (Fig. 3a). Root-nitrogen levels also did not differ significantly between control plants and plants exposed to foliar herbivory (Table 1). Independent of the treatment, nitrogen levels were significantly lower in secondary than in primary roots (Table 1). Foliar herbivory significantly affected the levels of indole glucosinolates (neoglucobrassicin and glucobrassicin) in the roots (Table 1). In presence of foliar herbivory, the levels of neoglucobrassicin (Fig. 4a) and glucobrassicin (Fig. 4b) significantly increased in both primary and secondary roots. Independent of the treatment, levels of neoglucobrassicin and glucobrassicin were significantly higher in secondary roots compared with primary roots. Concentrations of gluconasturtin (Fig. 4c) and sinigrin (Fig. 4d) did not differ significantly between the roots of control plants and plants exposed to foliar herbivory (Table 1). Independent of the treatment, gluconasturtin levels were significantly higher in primary than in secondary roots. Sinigrin levels did not differ significantly between primary and secondary roots.
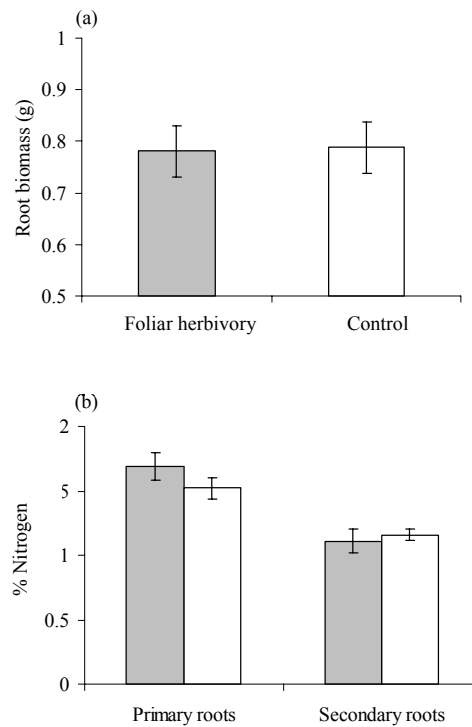
Figure 3. Mean (± SE) root biomass (a) and % of nitrogen in primary and secondary roots (b) of *Brassica nigra* plants exposed to foliar herbivory (by *P. brassicae*) (shaded bars), and control plants (open bars).

Table 1. Approximate F-test (F and P values) for the fixed effects and variance component estimates from REML (restricted maximum likelihood), analysis of the effect of foliar herbivory (by *P. brassicae*) and root-type on nitrogen and glucosinolate levels of *B. nigra* roots.

Factors	Nitrogen		Neoglucobrassicin		Glucobrassicin		Gluconasturtin		Sinigrin	
	F	P	F	P	F	P	F	P	F	P
Foliar herbivory (1)	1.6	0.2	15.0	<0.001	15.3	<0.001	0.5	0.4	1.7	0.2
Root - type (2)	28.4	<0.001	28.5	<0.001	89.5	<0.001	4.57	0.03	0.001	0.9
Interaction (1.2)	0.4	0.5	2.9	0.08	0.2	0.5	0.7	0.3	1.4	0.2
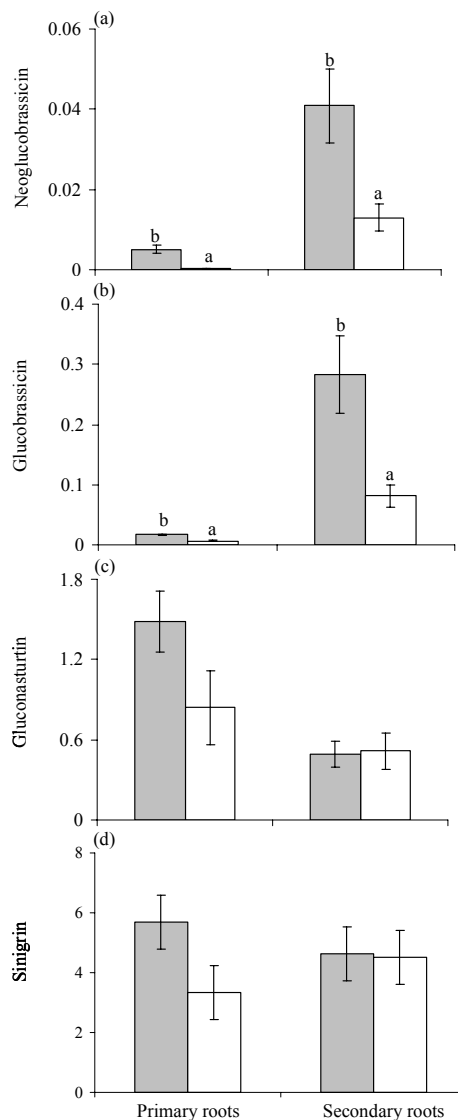
Figure 4. Mean (± SE) levels of glucosinolates (µmol g^{-1} dry mass) (a: neoglucobrassicin, b: glucobrassicin, c: gluconasturtin, d: sinigrin) in primary and secondary roots of *Brassica nigra* plants exposed to foliar herbivory (by *P. brassicae*) (shaded bars), and control plants (open bars). Within each root type, significant differences ($P < 0.05$) between treatments are indicated by different letters.

DISCUSSION

This study provides evidence that foliar-feeding insects, via systemic changes in the root quality of the shared host plant, can influence the development of root-feeding insects sharing the host plant. Moreover, the effects can be carried over

at least one trophic level higher, affecting the performance of a parasitoid. We found that foliar herbivory was significantly correlated with a reduction in successful development of *Delia radicum* and *Trybliographa rapae*. Survival of the herbivore and its parasitoid was two to three times higher on plants that were undamaged aboveground, compared with plants that were previously exposed to foliar herbivory. In addition, newly emerged adult *D. radicum* and *T. rapae* were also significantly smaller when the insects developed on plants that were initially damaged by foliar herbivores, compared with control plants. Several authors have argued that body size is the main target of selection in parasitoids and other insects, because it is correlated with life-history and demographic characters such as searching and mating efficiency, longevity and fecundity (Godfray 1994, Harvey et al. 1994, Bezemer and Mills 2003, Harvey 2005). Consequently, our results reveal that *D. radicum* and *T. rapae* not only suffer from increased mortality on plants previously exposed to foliar herbivory compared with control plants, but that fitness of the surviving adults is also compromised.

In a recent study based on the same multitrophic system, we reported that root herbivory by *D. radicum* reduced the performance of the foliar feeder *P. brassicae* (Soler et al. 2005). Several other studies have found no significant relationship between root herbivory and the performance of aboveground herbivores (e.g. Moran and Whitham 1990, Salt et al. 1996, Hunt-Joshi and Blossey 2005), whereas others found a positive relationship (e.g. Masters and Brown 1992). These results clearly show that the effects of belowground herbivores on their aboveground counterparts are likely to be association-specific. However, this contrasts with the rather consistent negative effect thus far observed in most reciprocal studies of aboveground herbivory on belowground insects (this study, Moran and Whitham 1990, Masters and Brown 1992, Salt et al. 1996, Tindall and Stout 2001, Hunt-Joshi and Blossey 2005). More work is clearly required in order to elucidate possible patterns in interactions between aboveground and belowground systems, and to determine if there are any clear patterns that emerge from them.

We also found that the amount of root biomass did not differ significantly between the foliar-damaged and control plants, suggesting that the negative effect of aboveground herbivory by *P. brassicae* on the development of *D. radicum* and *T. rapae* was based on qualitative, rather than quantitative changes in the root system. Because only three young caterpillars were allowed to feed from the foliage for a limited period of time, the physical amount of shoot tissue removed was negligible (R. Soler, personal observation). Changes in root biomass as a result of source-sink relationships within the plant are likely to occur when aboveground herbivore damage is much more extensive (Hunt-Joshi and Blossey 2005). Root nitrogen levels were also not significantly affected by foliar herbivory, and thus it is highly unlikely that changes in this important (and often limiting) primary plant compound were responsible for the observed effects.

On the other hand, levels of indole glucosinolates were significantly increased by about 30 % in plants that were exposed to foliar herbivores. Glucosinolates and their breakdown products, including indoles, have been shown to be involved in reducing plant quality for both generalist and specialist insect herbivores and nematodes (Li et al. 2000, Van Dam et al. 2005). Although indoles constituted only a small fraction of total glucosinolates in the plant (with sinigrin being by far the most prevalent), studies with other secondary compounds have shown that only trace amounts in the diet are required to exert strong effects on the development of herbivores and their parasitoids (Barbosa et al. 1986). Consequently, the negative relationship between aboveground herbivory and the development of *D. radicum* may be, at least partially, correlated with increased levels of indole glucosinolates in the roots of the shared host plant that were induced by feeding damage from *P. brassicae* larvae aboveground.

It is well-established that allelochemicals in the herbivore's diet can exhibit negative effects on the morphology, development, size and survival of their predators and parasitoids (reviewed by Harvey 2005, Ode 2006). *T. rapae* is

a koinobiont parasitoid of *D. radicum* and therefore, after being parasitised, *Delia* larvae continue feeding and growing until the final larval instar has been reached. At this time the developing parasitoid larvae begin destructive feeding and exponential growth (Harvey et al. 1994). At the end of the host's larval development, allelochemicals that may be present in the hemolymph and other tissues, such as fat body, are rapidly consumed by the koinobiont parasitoid (Harvey 2005). Due to the tight linkage between host and koinobiont parasitoid development, in our study, increased mortality and reduced adult size observed in the *T. rapae* adults on plants exposed to foliar herbivory, could probably be attributed to a corresponding increased mortality and reduced size of its host *D. radicum*. Parasitoids, in particular, are highly susceptible to very small changes in the quality of the host's internal biochemical environment (Harvey 2005). Other studies have shown that parasitoids can be more affected than their hosts by the quality of the host plant (Harvey et al. 2003, Soler et al. 2005). This appears not to be the case in our studied trophic chain, since we found comparable effects of foliar herbivory on the root herbivore and its parasitoid.

In summary, this study has reported that herbivore damage of plant shoots can have significant negative effects on the development of belowground herbivores and their natural enemies. Combined with the results of an earlier study based on the same system, we have demonstrated that these effects are largely reciprocal (Soler et al. 2005). Our research further highlights the importance of integrating the belowground domain with aboveground systems in order to better understand the myriad of factors that shape the evolution, assembly and functioning of communities and ecosystems (Wardle et al. 2005). Studies over larger spatial scales have already shown that aboveground herbivores can influence the community structure of the belowground system and vice-versa (De Deyn and Van der Putten 2005, Wardle et al. 2004). Further studies, combining interactions between aboveground and belowground communities in a multitrophic framework, and which seek to explore both processes and mechanisms, are required if we are to develop a better

understanding of the many complex factors that influence community structure and function.

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Chapter 4

Root herbivores influence the behaviour of an aboveground parasitoid through changes in plant-volatile signals

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SUMMARY

It is widely reported that plants emit volatile compounds when they are attacked by herbivorous insects, which may be used by parasitoids and predators to locate

their host or prey. The study of herbivore-induced plant volatiles and their role in mediating interactions between plants, herbivores and their natural enemies have been primarily based on aboveground systems, generally ignoring the potential interactions between above and belowground infochemical- and food webs. This study examines whether herbivory by *Delia radicum* feeding on roots of *Brassica nigra* (black mustard) affects the behaviour of *Cotesia glomerata*, a parasitoid of the leaf herbivore *Pieris brassicae*, mediated by changes in plant volatiles. In a semi-field experiment with root-damaged and root-undamaged plants *C. glomerata* prefers to oviposit in hosts feeding on root-undamaged plants. In addition, in a flight-cage experiment the parasitoid also prefers to search for hosts on plants without root herbivores. Plants exposed to root herbivory were shown to emit a volatile blend characterised by high levels of specific sulphur volatile compounds, which are reported to be highly toxic for insects, combined with low levels of several compounds, i.e. beta-farnesene, reported to act as attractants for herbivorous and carnivorous insects. Our results provide evidence that the foraging behaviour of a parasitoid of an aboveground herbivore can be influenced by belowground herbivores through changes in the plant volatile blend. Such indirect interactions may have profound consequences for the evolution of host selection behaviour in parasitoids, and may play an important role in the structuring and functioning of communities.

INTRODUCTION

The defence of plants against insect herbivores involves different strategies. Plants can defend themselves directly through the production of morphological structures on the leaf surface (i.e. trichomes) that deter herbivore colonization, and by producing or increasing levels of toxic compounds that deleteriously affect the development of phytophages (Dicke and Van Loon 2000). The plant defence system can also involve indirect mechanisms, including the production

and release of plant volatile compounds as a response to herbivore feeding, which attract natural enemies (i.e. parasitoids and predators) of the attacking herbivores (Vet and Dicke 1992, Dicke 1999, Vet 1999). The role of herbivore-induced volatiles in mediating interactions between plants, herbivores and their natural enemies has received considerable attention over many years (Price et al. 1980, Turlings et al. 1990). Thus far, most studies have been primarily based on aboveground interactions, but similar plant-induced indirect defense responses have also been observed belowground (Van Tol et al. 2001, Rasmann et al. 2005).

It is known that root-associated organisms can affect the development of leaf associated herbivores sharing the host plant (Gange and Brown 1989, Masters 1995, Van der Putten et al. 2001, Bezemer et al. 2003), and higher trophic levels including parasitoids, and even hyperparasitoids of the fourth trophic level (Soler et al. 2005). Recently, there has been a growing interest in how soil organisms, such as root-feeding insects, nematodes, or arbuscular mycorrhizal fungi, may also influence the levels of parasitism of aboveground herbivores (Masters et al. 2001, Gange et al. 2003, Bezemer and Van Dam 2005, Bezemer et al. 2005, Poveda et al. 2005). There is increasing evidence that the effect of root-associated organisms on the development of the aboveground trophic chains (herbivores-parasitoids-hyperparasitoids) is largely mediated by changes in quantity and/or quality of the shared host plant (reviewed by Bezemer and Van Dam 2005). However, little is known about the influence of root-associated organisms on the behaviour of parasitoids of aboveground herbivores and the mechanisms mediating the interactions.

Recently, Guerrieri et al. (2004) showed that the behaviour of aboveground parasitoids can be affected by arbuscular mycorrhizal fungi. In absence of their hosts, aphid parasitoids were strongly attracted to mycorrhizal plants over conspecific plants without mycorrhizal association in the roots (Guerrieri et al. 2004). Although studies are scarce, there is also some evidence that soil organisms can influence indirect plant defense responses aboveground

(Wäckers and Bezemer 2003, Bezemer and Van Dam 2005). For example, Rasmann et al. (2005) showed that root herbivory induces a volatile signal in the soil that attracts entomopathogenic nematodes, whilst simultaneously inducing the release of the same volatile compound aboveground from the leaves of the plant. However, the consequences of such belowground defence induction for the behaviour of aboveground natural enemies remain unexplored. Further studies integrating associations between plants, insect herbivores, and their natural enemies aboveground with the biological processes that occur in the soil, and the mechanisms mediating the interactions, are crucial for a better understanding of interactions between plants and other organisms in a multitrophic context.

Recently we showed that *Cotesia glomerata* (Hymenoptera: Braconidae), an aboveground parasitoid of caterpillars of the cabbage butterfly, *Pieris brassicae* (Lepidoptera: Pieridae) developed significantly slower and adults were smaller when roots of *Brassica nigra* (Brassicaceae) plants were damaged by larvae of the cabbage root fly, *Delia radicum* (Diptera: Anthomyiidae) (Soler et al. 2005). These negative effects appeared to be driven by increases in foliar levels of allelochemicals induced by the root-feeding insects (induced direct defense). Parasitoid females are known to be well adapted to exploit chemical information coming from the host plants to select the most profitable host for their off-spring (Godfray 1994, Dicke 1999). Considering that the performance of *C. glomerata* was significantly reduced when developing in hosts feeding on plants exposed to root herbivory, we tested whether *C. glomerata* uses cues induced by belowground herbivores to avoid root-damaged plants when suitable oviposition sites are not limiting.

We tested the hypothesis that there is a clear preference-performance correlation in the parasitoid, i.e. that females will preferentially search for and parasitize hosts on root-undamaged plants, where their progeny will develop most successfully. We examined the effects of root herbivory on the plant volatile blend, to correlate with our behavioural data. We specifically address the following questions: (1) is host acceptance of the parasitoid affected by

belowground herbivory? (2) Is plant preference of the parasitoid affected by belowground herbivory? If so, does it differ when exposing the plants to different *Delia* larval stages? And (3) if variation in the parasitoid behaviour is observed, can it be attributed to changes in the plant volatiles triggered by root herbivory?

MATERIAL AND METHODS

Our study is based on interactions involving a naturally occurring system in western Europe. Black mustard, *Brassica nigra* (Brassicaceae), is a widely distributed annual crucifer that is common along rivers in the Netherlands (Schaminée et al. 1998). *Brassica nigra*, like other members of the Brassicaceae, possesses potent inducible direct and indirect defenses via the production of glucosinolates and their breakdown products, providing excellent potential for studying multitrophic interactions (Feeny and Rosenberry 1982, Traw and Dawson 2002, Van Dam et al. 2003). It is attacked by several aboveground and belowground specialist herbivores, including the large cabbage white butterfly, *Pieris brassicae* (Lepidoptera: Pieridae) whose larvae feed on the shoots and flowers of this and related species (Harvey et al. 2003). *Cotesia glomerata* is a gregarious endoparasitoid that attacks young larvae of several species of pierid butterflies; with *P. brassicae* as its preferred host in Europe (Feltwell 1982). To locate their hosts, *C. glomerata* females rely on infochemicals both from the plant and its host (Geervliet 1997). Mustard plants are also attacked by specialised root-feeding herbivores, including the cabbage root fly, *Delia radicum* (Diptera: Anthomyiidae) (Coaker and Finch 1971). Females lay eggs on the soil surface around the plant stem, and the emerged larvae feed on the plant roots causing rapid infection by microorganisms. *D. radicum* has associated gut microbial symbionts, which may be bacteria, yeast or protozoa that are assumed to enrich the nutritional value of the food by providing essential vitamins and amino acids or by digesting refractory plant materials. However, little is known

about its digestive biochemistry, the source of these organisms and the maintenance between generations (Lukwinski et al. 2006). It is important to notice then, that for this system, the effects of root herbivory cannot be fully separated from the effects of the microorganisms associated to *Delia radicum*.

Pieris brassicae (leaf herbivore) was obtained from an insect culture maintained at the Laboratory of Entomology of Wageningen University, the Netherlands and was cultured on *Brassica oleracea* plants. *Cotesia glomerata* (parasitoid) and *Delia radicum* (root herbivore) were obtained from cultures maintained at the Netherlands Institute of Ecology, Heteren, The Netherlands. The root herbivore was cultured on *Brassica napus* roots. *Brassica nigra* seeds were collected from a single population in the northwest of Wageningen, the Netherlands. Seeds were surface sterilised and germinated on a bed of glass-pearls (pearls of 1 mm diameter). One week after germination seedlings were transplanted into 1.2 l pots. The plants were grown in a greenhouse, at $22 \pm 1^{\circ}$C (day) and $16 \pm 1^{\circ}$C (night), 70% RH and 16:8 h day:night. Natural daylight was supplemented by metal-halide lamps (225 μmol s^{-1} m^{-2} PAR). Plants were watered daily; only non-flowering plants were used in the experiments.

Host-acceptance in the semi-field experiment

In a two-choice experiment with 26 plants we examined if *C. glomerata* females prefer to parasitize their larval hosts (*P. brassicae*) feeding on root-undamaged plants over hosts feeding on plants of which roots were fed on by *D. radicum*. The experiment was performed outdoors (July 2005) in a transparent tent (8 × 4 × 2.5 m). On 13 plants, eight third instar (L3) *D. radicum* larvae per plant were introduced six days before the test (root herbivory treatment), while the 13 other plants were kept root-undamaged and served as control. One day prior to the test, all plants were infested with 10 L1 *P. brassicae* larvae. Each brood was placed on the youngest fully developed leaf of each plant. Plants of similar height and shape were used to avoid any potential effects of plant characteristics on parasitoid behaviour.

The following day, all plants were placed in the tent alternating root-damaged and root-undamaged plants. The distance between plants was approximately 1 m. Ten *C. glomerata* females were then released into the tent, allowed to forage freely during two h and then recaptured. Subsequently, the *P. brassicae* larvae were harvested and dissected to determine the number of parasitised and healthy larvae per plant. Parasitism was identified by the presence of parasitoid eggs, using a stereo-microscope. The number of plants with parasitised larvae was determined, and the percentage of root-damaged and root-undamaged plants selected by the parasitoid females calculated. In addition, the number of parasitised caterpillars per plant on which parasitised larvae were found was recorded.

Plant-preference in the flight-cage experiment

We used a flight-cage, comparable to a wind tunnel described in Geervliet et al. (1994), to examine if *C. glomerata* discriminates between plants fed on by *Delia* (root-damaged plant) and root-undamaged plants. A cage of 1.2 × 1.0 × 0.8 m, with nets at the front and back and otherwise of plexiglas, was placed on a table inside a transparent cloth tent in a greenhouse compartment, at 22 ± 1^oC and 70% RH. Plant preference was tested in a two-choice set-up. We performed the following choice-test comparisons: control test: undamaged plants vs *Pieris* (host caterpillar)-infested plants; test a: undamaged plants vs plants with roots damaged during the entire *Delia* larval development time (from L1 to L3); test b: undamaged plants vs plants with roots damaged by *Delia* L3 larvae; test c: undamaged plants vs plants with roots damaged by *Delia* L1-L2 larvae; test d: plants with *Pieris* feeding on it vs plants with *Pieris* feeding on it and roots damaged by *Delia* L3 larvae (see detailed description below). For each choice test 10 pairs of plants were used, and the distance between the plants within a plant pair was 0.70 m. For each plant pair, 10 to 12 parasitoid females of five to eight days old were released from a distance of 1 m from the plants. A choice was recorded when the parasitoid alighted on the foliage of a plant. Each choice

test was terminated after choices of 10 females were registered. Overall, 76% of the female wasps actively made a choice, independent of the choice test, adding to a total of approximately 100 *C. glomerata* females observed per comparison. In order to increase the parasitoid motivation to search, one first instar larva of *P. brassicae* was presented to each female wasp for oviposition immediately prior to each test (Vet et al. 1995). The offered first instar larvae of *P. brassicae* had been feeding on *Brassica oleracea*, a different host plant as presented in the test, to prevent a possible associate learning-induced bias in the plant choice by *C. glomerata* (Vet et al. 1995). Experimental plants exposed to root herbivory were checked after the test to ensure that *D. radicum* larvae were present and actively feeding. More than 80% of the larvae that were introduced were recovered in each test. To avoid the interference between plant-odours of different replicates or treatments, the air inside the cage was ventilated between tests using a fan.

Control test

Undamaged plants vs *Pieris*-infested plants (control vs LH). Previous studies reported that *C. glomerata* is significantly more attracted to *Brassica* plants infested by its hosts (*P. brassicae*) than to uninfested plants (Steinberg et al. 1993). Therefore, to test our flight-cage set-up, we initially compared plant preference of *C. glomerata* between *B. nigra* plants with and without a brood of 10 first instar larvae of *P. brassicae*. Approximately 24 h before the test, the *P. brassicae* larvae were placed on the third new leaf of the plants, in a clip-cage of 5.5 cm diameter. Clip-cages were similarly placed on the control plants without *P. brassicae* larvae. The clip-cages were removed prior to the choice tests to fully expose the larvae to the parasitoids.

Test a, b, c

Undamaged plants vs plants with root herbivores (control vs different larval stages of RH). After checking the flight-cage set-up, parasitoid preference was compared between *B. nigra* plants without (control) and with *D. radicum* larvae

(root herbivory treatment, performed by different larval stages). In all cases the *Delia* larvae were carefully placed with a brush next to the stem of the plant at the soil surface. Test a (L1-L3): eight *D. radicum* larvae were introduced as first instar (L1) and the test was performed when larvae had reached the end of the last (third) instar (L3), being approximately the entire larval developmental time (approximately 15 days). Test b (L3): damage caused by late instar larvae only: eight early-stage L3 *D. radicum* larvae fed during six days and the test was performed at the end of L3. Test c (L1-L2): damage caused by early instar larvae only: eight L1 *D. radicum* fed for nine days, and thus the test was performed when larvae were L2. Due to early flowering of some plants with *Delia*, five replicate plants instead of 10 were used for test c.

Test d

Plants with *Pieris* vs plants with *Pieris* and roots damaged by *Delia* L3 larvae (LH vs LH + RH$_{L3}$). *P. brassicae* larvae were placed on the plants 24 h before the test, following the same procedure described above in the control tests. Root herbivory was similar as in test b.

Plant volatiles

Volatiles were collected from plants exposed to root herbivory (RH), leaf herbivory (LH), both types of herbivory (LH + RH), or no herbivory (control). The entire plants (including the pots and soil) were placed in the chambers to collect the volatiles. There were six replicate plants per treatment. Plants of similar size were used for the volatile collection. *Delia* infested plants were inoculated with eight L3 *D. radicum* larvae six days prior to the collection of volatiles. Plants with leaf herbivory were inoculated with 10 L1 *P. brassicae* larvae 24 h prior to volatile collection. Plants were transferred to four separate 17 l glass bell-shaped collection chambers that had been placed in a controlled climate cabinet (21°C, 70% RH). The chambers were constantly supplied at the top with 300 ml of pressurised air (Hoekloos, the Netherlands) and were cleaned

over a Zero Air generator to remove hydrocarbons (Parker Hannifin Corp, Tewksbury, MA, USA). Volatiles were collected in a steel trap filled with 150 mg Tenax TA and 150 mg Carbopack B using a vacuum pump. Collection flow rates were set to 100 ml min^{-1}. After one h the traps were removed from the pump and capped until analysis. In this way, we measured six full series of four plants and two background profiles from an empty glass chamber within one day.

Volatiles were desorbed from the traps using an automated thermodesorption unit (model Unity, Markes, Pontyclun, United Kingdom) at 200°C for 10 min (He flow 30 ml min^{-1}) and focused on an cold trap (-10°C). After 1 min of dry purging, trapped volatiles were introduced into the GC-MS (model Trace, ThermoFinnigan, Austin, Texas, USA) by heating the cold trap for 3 min to 270°C. The split rate was set to 1:4 and the column used was a 30 m × 0.32 mm ID RTX-5 Silms, film thickness 0.33 μm. Temperature program: from 40°C to 95°C at 3°C min^{-1}, then to 165°C at 2°C min^{-1}, and finally to 250°C at 15°C min^{-1}. The volatiles were detected by the MS operating at 70 eV in EI mode. Mass spectra were acquired in full scan mode (33-300 AMU, 3 scans s^{-1}). Compounds were identified by their mass spectra using deconvolution software (AMDIS) in combination with Nist 98 and Wiley 7th edition spectral libraries and by comparing their linear retention indices. Additionally, mass spectra and/or linear retention indices were compared with values reported in the literature, obtained by interpolating homologous series, or by analyzing reference substances (farnesene, benzonitrile, dimethyldisulfide, dimethyltrisulfide and limonene; Sigma-Aldrich, Zwijndrecht, NL). Identified peaks in the chromatogram were integrated by Xcalibur software (version 1.3, Finnigan). To exclude potential interference by co-eluting compounds, specific quantifier ions were carefully selected for each individual compound of interest. In general, these quantifier ions were similar to the most intense model ions extracted from the raw mass spectrum by AMDIS. The integrated absolute signal of the quantifier ion(s) were used for comparison between the treatments. Peak areas in each sample were divided by the total volume in ml that was sampled in the trap,

to correct for small differences in sampling time and flow rates over individual traps.

Statistical analyses

For the host-acceptance semi-field experiment, proportion of plants with parasitization between control plants and plants exposed to root herbivory were compared using a two-sample binomial test. Percentages of parasitism per plant with parasitism were arc-sine transformed and tested using one-way ANOVA. Normality and homogeneity of variance were checked by inspection of the residuals after model fitting. For the plant preference flight-cage experiments, a binomial test was used to determine whether plant preferences of the wasps differed significantly from a non-preference situation ($p = q = 0.5$, two-tailed, $\alpha = 0.05$).

A total of 249 compounds were registered in the volatile blends of plants exposed to the different treatments. A subset of 26 compounds belonging to the isothiocyanates, monoterpenes, sesquiterpenes, terpenes, nitric compounds, sulfur and other/aromatic compounds were selected for detailed analysis, as they are known to play a role in plant-insect interactions in cruciferous plants (Reddy et al. 2002, Ibrahim et al. 2005, Kappers et al. 2005). Differences and similarities in the volatile blends between treatments were analysed by canonical discriminant analysis (CDA) (McGarigal et al. 2000). CDA is a dimension-reduction technique similar to principal component analysis which calculates linear combinations of the original variables (i.e. volatile compounds). The linear combinations, usually referred to as canonical variates, summarize the information contained in the original variables and maximize between-treatment variation. This allows for the identification of those compounds that discriminate most clearly between the treatments. To visualize multivariate similarities in volatile blend among the treatments, we plotted the location of each sample with respect to their position on the first and second canonical variates (CDA plot). To quantify the relative (dis)similarity between treatments in volatile blends we

calculated Mahalonobis distances which measure the distance between treatment-centroids in multivariate space (McGarigal et al. 2000). We used standardised canonical coefficients to assess the relative contribution of each compound to the canonical variates. For each compound separately we tested the effect of leaf herbivory (LH), root herbivory (RH) and the interaction (LH×RH) using analysis of variance on log-transformed peak areas. Normality, independence and homogeneity of variance were checked for each variable by inspection of the residuals after model fitting. For some variables (beta-farnesene, dimethyl disulfide and dimethylnonatriene) a non-parametric test was used (Kruskal-Wallis) since their distribution could not hold the assumptions for a standard parametric analysis of variance.

RESULTS

Host-acceptance in the semi-field experiment

Root herbivory significantly affected the oviposition behaviour of *C. glomerata*. Parasitised hosts were found on 85% of the control plants, compared to 46% on plants with root herbivory ($Z = 2.06$; $P = 0.03$). When parasitization had occurred on a plant, the percentage of parasitism between plants with and without root herbivory did not differ (control: 57 ± 10; root herbivory: 83 ± 13; $F_{1,15} = 2.55$; $P = 0.13$).

Plant-preference in the flight-cage experiment

C. glomerata showed a clear preference for *B. nigra* plants damaged by the caterpillar host *P. brassicae*, as compared to plants without the leaf herbivore ($Z = 5.58$; $P < 0.0001$; data not shown). Eighty percent of the released wasps alighted on plants that were infested with their host larvae, confirming that the flight cage set-up was appropriate to measure the plant preference of the parasitoid.

In absence of the caterpillars, root herbivory significantly affected plant preference of the parasitoid, and the effect differed with root herbivory intensity (Fig. 1a-c). Parasitoids showed a clear preference for root-undamaged *B. nigra* plants and generally avoided plants exposed to root herbivores feeding on the roots during almost the entire larval development time (L1-L3: Fig. 1a) (Z = 2.40; P = 0.01). Similarly, parasitoids clearly preferred root-undamaged plants to plants that had been exposed to root herbivores feeding on the roots during the final larval instar (L3: Fig. 1b) (Z = 2.81; P = 0.004). However, the parasitoid did not avoid *B. nigra* plants that had solely been exposed to early instar larvae of the root herbivores (L1-L2: Fig. 1c) (Z = 0.1; P = 0.92). Plant preference of the parasitoid was also not significantly affected by root herbivory when its host *P. brassicae* was present on the leaves of both root-damaged and root-undamaged plants (Fig. 1d), with approximately fifty percent of the released female parasitoids alighting on each plant choice (Z = 0.49; P = 0.62). The response time, defined as the time between release of the parasitoid and the parasitoid landing on the plant, was similar in all the experiments and did not depend on the choice offered ($F_{4,40}$ = 1.79; P = 0.14).
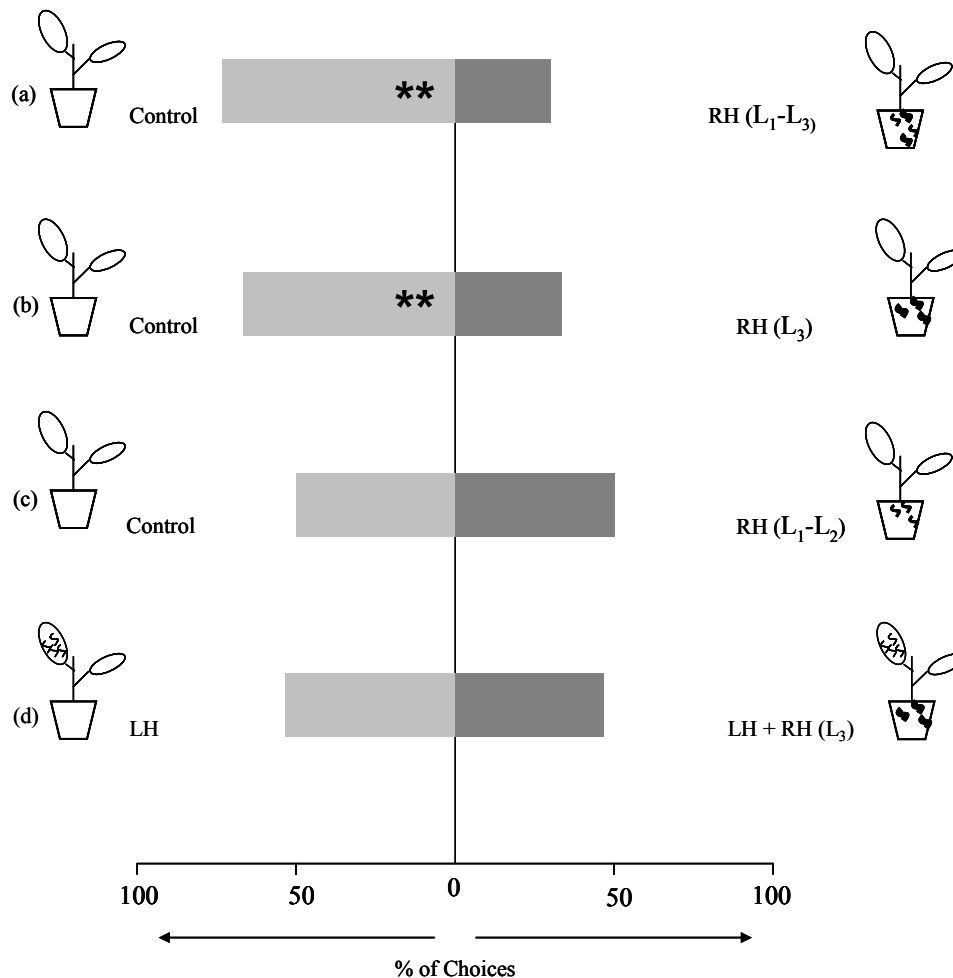
Figure 1. Percentage of choices of *C. glomerata* females, in the two-choice experiment, between plants exposed to root herbivory (RH) [(by the entire larval root fly development time: L1-L3); (by late instar root fly larvae: L3); (by young instar root fly larvae: L1)], leaf herbivory (LH), both types of herbivory (LH+RH), or no herbivory (control). Root herbivory was caused by *D. radicum* larvae and leaf herbivory was caused by the parasitoid's host larva *P. brassicae.* Asterisks indicate significant preferences within tests (**: $P \leq 0.01$).

Plant volatiles

Using GC-MS technique we examined the volatile blend of plants exposed to root (RH) or leaf herbivory (LH), both types of herbivory (LH+RH), or no herbivory (control). The results of the canonical discriminant analysis show a clear separation of the four treatments based on the volatile blends (Fig. 2). The first and second axis explained 67% and 22% of the total variation in volatile compounds, respectively. The first axis clearly discriminated between LH and LH+RH plants on the one hand, and RH and control plants on the other hand

(Fig. 2). The second axis mainly separated RH from control plants (Fig. 2). The LH+RH and the LH group of plants partially overlapped, and the similarity in volatile blends between these two groups is also evidenced by the small Mahalonobis distance between them, compared to the higher distance recorded between RH and control plants (Fig. 2). The terpenes beta-farnesene and dimethylnonatriene showed the closest correlation with the first canonical variate (highest values for score 1 in Table 1) and were both negative. LH and LH+RH plants had negative canonical coefficients for the first axis, implying that the volatile blends of the LH and the LH+RH plants were characterised by a higher amount of these two compounds than the RH and control plants. The second axis was strongly determined by the sulfur compounds dimethyl disulfide and dimethyl trisulfide (Table 1). In the second axis, RH plants had negative coefficient values and control plants had positive coefficient values. High negative values were then associated with higher levels of these compounds, which was the case for the RH plants. The pattern showed by the CDA is consistent with the analysis of variance, which showed that beta-farnesene and dimethylnonatriene were significantly higher in plants exposed to leaf herbivory, and dimethyl disulfide and dimethyl trisulfide were the two volatile compounds significantly higher and associated with plants exposed to root herbivory (Table 1).
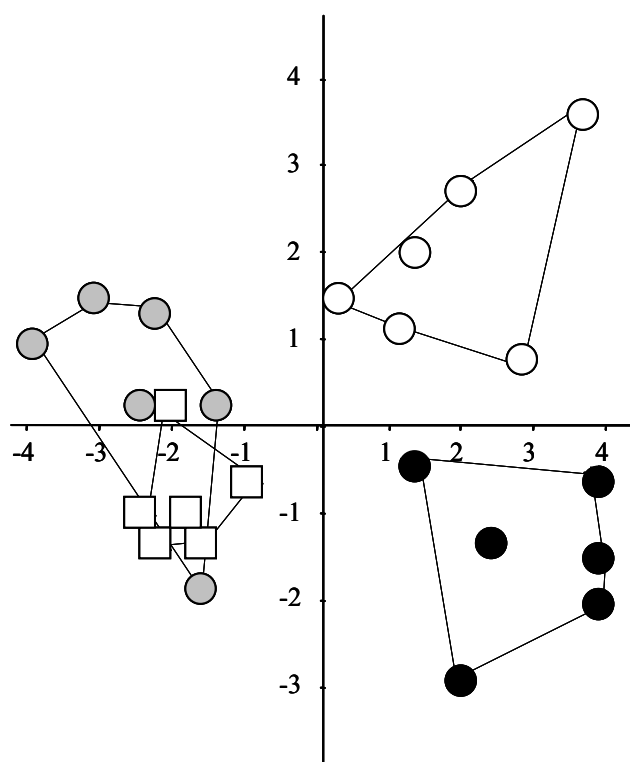
		Mahalonobis distances			
☐	LH+RH	0			
○	Control	21	0		
●	RH	22	12	0	
◐	LH	2	21	32	0
		LH+RH	Control	RH	LH

Figure 2. Two dimensional canonical discriminate analysis (CDA) plot of the volatile blends of plants exposed to root herbivory (black circles, RH) or leaf herbivory (grey circles, LH), no herbivory (white circles, control) or both types of herbivory (white squares, LH+RH). Each point represents the volatile blend of a sampled plant. Below, Mahalonobis distances between the volatile blends are presented.

Table 1. Log transformed peak area (mean ± SE) of volatile compounds, standardised canonical coefficients for the first two axes of the CDA plot (score 1 and 2) and two-way analysis of variance (Kruskal-Wallis -non parametric-analysis of variance for linalool, beta-farnesene and dimethyl disulfide) for individual volatile compounds of *Brassica nigra* plants exposed to root (RH) or leaf herbivory (LH), both types of herbivory (LH+RH), or no herbivory (Control). Root herbivory was caused by *D. radicum* larvae and leaf herbivory was caused by *P. brassicae* larvae. Non significant P-values (P > 0.05) are indicated by ns.

Volatiles	Control	LH	RH	LH + RH	Score 1	Score 2	p (LH)	p (RH)	p (LH×RH)
alpha-farnesene	2.79 ± 0.63	2.84 ± 0.57	2.87 ± 0.64	3.81 ± 0.26	-0.073	-0.122	ns	ns	ns
alpha-humulene	1.90 ± 0.61	1.68 ± 0.75	1.98 ± 0.88	2.13 ± 0.68	0.0004	0.077	ns	ns	ns
alpha-muurolene	2.56 ± 0.53	2.79 ± 0.57	2.57 ± 0.52	2.20 ± 0.70	-0.023	0.058	ns	ns	ns
alpha-pinene	3.30 ± 0.67	2.87 ± 0.68	3.31 ± 0.66	3.38 ± 0.69	0.025	0.008	ns	ns	ns
alpha-ylangene	3.40 ± 0.15	3.61 ± 0.07	3.58 ± 0.18	3.02 ± 0.61	0.055	0.018	ns	ns	ns
aromadendrene	1.13 ± 0.51	2.06 ± 0.44	1.25 ± 0.57	1.33 ± 0.60	-0.076	0.001	ns	ns	ns
benzene	1.73 ± 0.78	1.94 ± 0.62	1.59 ± 0.71	0.53 ± 0.53	-0.040	0.087	ns	ns	ns
benzonitrile	0.98 ± 0.62	1.93 ± 0.61	1.09 ± 0.70	0.94 ± 0.59	0.020	0.021	ns	ns	ns
beta-cubebene	0.52 ± 0.52	1.06 ± 0.67	1.70 ± 0.76	0.94 ± 0.60	-0.057	-0.148	ns	ns	ns
beta-farnesene	0.00 ± 0.00	2.59 ± 0.53	0.54 ± 0.54	1.82 ± 0.82	-0.280	-0.133	0.004	ns	ns
2-beta pinene	2.27 ± 0.72	2.62 ± 0.54	3.38 ± 0.16	3.24 ± 0.07	0.027	-0.294	ns	ns	ns
3-butenenitrile	1.33 ± 0.84	2.08 ± 0.93	0.66 ± 0.66	1.42 ± 0.89	-0.069	0.066	ns	ns	ns
cadinene	1.38 ± 0.62	1.36 ± 0.61	1.46 ± 0.65	0.46 ± 0.46	0.042	0.082	ns	ns	ns
camphene	3.07 ± 0.13	2.97 ± 0.27	3.19 ± 0.16	3.33 ± 0.13	-0.089	-0.157	ns	ns	ns
delta-3-carene	2.71 ± 0.56	3.33 ± 0.20	2.87 ± 0.59	3.46 ± 0.20	-0.086	-0.090	ns	ns	ns
dimethyl disulfide	0.00 ± 0.00	0.00 ± 0.00	1.72 ± 1.09	2.42 ± 1.08	0.012	-0.353	ns	0.01	ns
dimethyl trisulfide	1.54 ± 0.69	2.73 ± 0.57	3.43 ± 0.18	3.34 ± 0.11	-0.095	-0.474	ns	0.01	ns
dimethylnonatriene	0.79 ± 0.79	5.17 ± 0.15	1.48 ± 0.93	5.12 ± 0.21	-0.499	-0.293	<0.0001	ns	ns
junipene	3.29 ± 0.13	3.27 ± 0.11	3.33 ± 0.17	3.29 ± 0.10	0.088	-0.033	ns	ns	ns
limonene	3.24 ± 0.66	3.91 ± 0.20	4.09 ± 0.17	3.99 ± 0.21	0.003	-0.269	ns	ns	ns
linalool	0.00 ± 0.00	1.58 ± 0.72	0.80 ± 0.50	0.84 ± 0.53	-0.115	-0.177	ns	ns	ns
m-cymene	1.11 ± 0.70	1.34 ± 0.85	0.59 ± 0.59	1.89 ± 0.84	-0.02	0.027	ns	ns	ns
methyl thiocyanate	1.08 ± 0.69	1.54 ± 0.69	0.56 ± 0.56	1.07 ± 0.67	-0.087	0.087	ns	ns	ns
thujone	1.74 ± 0.59	1.63 ± 0.73	2.25 ± 0.72	1.56 ± 0.70	0.082	-0.049	ns	ns	ns
trans-caryophyllene	4.09 ± 0.27	3.6 ± 0.55	2.7 ± 1.23	4.03 ± 0.46	0.046	0.046	ns	ns	ns
trimethyl tridecatetraene	2.53 ± 0.80	4.09 ± 0.11	2.97 ± 0.60	3.42 ± 0.69	-0.126	-0.051	ns	ns	ns

DISCUSSION

The results of this study show that root-feeding insects can influence plant preference and oviposition behaviour of a parasitoid of an aboveground herbivore sharing the host plant. The underlying mechanism points to changes in the volatile blend of the plant. Overall, the parasitoid *C glomerata* exhibited a clear preference for plants that had not been attacked by larvae of the root herbivore,

D. radicum. An earlier study (Soler et al. 2005) reported that the performance, i.e. the development time and pupal weight of *C. glomerata* is negatively affected by foliar increases in phytotoxins (direct defense) induced by the root herbivore *D. radicum*, sharing the host plant. In the present study we find that the parasitoid is able to recognize plants on the basis of the presence or absence of root herbivores, and prefers to search and lay their off-spring in 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 and Visser 1990, Godfray 1994). These models have been exclusively based on aboveground model systems, whereas our results suggest that *C. glomerata* females are also able to exploit root-induced signals to evaluate and select the most suitable host for its off-spring.

The volatile blend of plants differed depending on whether the plant was exposed to root herbivory, leaf herbivory (by the parasitoid's host) or was undamaged. 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 disulfide and dimethyl 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 for 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 plants.

In the plant preference flight-cage experiment *C. glomerata* strongly distinguished between plants with and without root herbivory (RH vs control), preferring to search for hosts on root-undamaged plants. The ability of *C. glomerata* to discriminate between plants with or without root herbivores was found to depend on the stage of *D. radicum* feeding on the root system. Parasitoids only exhibited a clear discrimination when final (L3) instars of *D. radicum* were present, as opposed to solely younger (L1, L2) instars. In most holometabolous insects (insects with defined feeding and reproductive stages), the vast majority of damage is incurred by the feeding larva during the final stadium, in some instances exceeding 90% (Slansky 1986). This may suggest that a critical level of root damage may be an important pre-requisite for the parasitoid to detect the presence of root herbivores, and to actively make foraging decisions on this basis. Alternatively, different root fly larval stages may have induced different chemical profiles in *B. nigra* plants. More studies are required to understand the mechanisms mediating this differentiation by the female parasitoid.

C. glomerata preferentially attacked caterpillars on plants where *D. radicum* larvae were absent. In the time allowed for foraging, hosts on 65% of the plants had parasitization, and more that two thirds of those plants were root-undamaged. It is known that parasitoids only spend certain amounts of time in host patches before 'moving on', depending on the profitability of the patch (Vet et al. 1995, Waage 1979, Vos et al. 1998). In a confined setting, it is likely that if the experimental time had been extended most or even all of the plants could have been visited and every host parasitised. Our aim was to allow the parasitoids to forage just long enough to determine whether plants with or without root herbivores were preferred.

Because many parasitoids, including *C. glomerata*, are quite specialised and have limited host ranges, they have unquestionably evolved highly efficient mechanisms to perceive chemical cues that are associated with high quality hosts (Geervliet et al. 1998). Our study provides clear evidence of this adaptive trait in

C. glomerata. However, when testing plant preference in the flight-cage, the parasitoid did not distinguish between root-damaged and root-undamaged plants when its host was present in both plants (LH vs LH+RH). The CDA plot (and the low Mahalonobis distance) clearly illustrate that the volatile blend of the root-damaged plants with hosts (LH+RH plants) is fairly similar and partially overlaps with the volatile blend of the root-undamaged plants with hosts (LH plants). It is possible that the enclosed environment of the flight-cage leads to less contrasting odour-sources which can affect plant preference by parasitoids (Mumm and Hilker 2005) or may have caused volatile interference. The latter may be expected when the volatile compounds of plants with hosts that are responsible for attraction of parasitoids show overlap with volatile compounds released by other plant/plant-herbivore association (Vos et al. 2001), and this may be enhanced in an enclosed environment. Alternatively, it is possible that *C. glomerata* females decided not to oviposit in hosts on plants with root herbivory, although they landed on them but we did not study this foraging decision. More studies are clearly required to further understand the indirect interactions between belowground organisms and parasitoids of aboveground herbivores, and the mechanisms mediating the interactions.

Previous studies showed that the influence of mutualistic (arbuscular mycorrhizal fungi) organisms as well as of root herbivores enhanced the intensity of aboveground tritrophic interactions, by increasing the percentage of parasitism of leaf herbivores feeding on plants exposed to soil organisms (Masters et al. 2001, Gange et al. 2003, Poveda et al. 2005). In our study we clearly show that influences of interactions in the soil domain may also result into completely different complex aboveground processes. Here, the decision of *C. glomerata* to avoid root infested plants points to a preference-performance correlation, since parasitoid off-spring performs better in root uninfested than in root infested plants (Soler et al. 2005).

It is important to note however, that for *D. radicum* and related species the effects of root herbivory can not be fully separated from the effects of

microorganisms directly and indirectly associated with them. It is reported that *D. radicum* possesses microorganisms in the alimentary tract that contribute to host nutrition directly as a food source as well as by providing increased digestive potential (Lukwinski et al. 2006). Moreover, the damage caused in the roots as a consequence of feeding provides a point of entry for subsequent infection by endemic root rot pathogens (Soroka et al. 2004). The gram-bacilli symbiotic bacteria found in the root herbivore gut as well as the rot root-associated microorganisms may also influence the plant defense system being responsible for inducing the release of certain compounds in the volatile blend of the plant, or might even directly emit specific volatile compounds. Little is know however, about interactions between root herbivores and their associated microorganisms, and the role that this may play in aboveground interactions. Further studies are required to better understand the mechanisms mediating these complex interactions.

Our study shows that root herbivores can influence the behaviour of a third trophic level aboveground, as mediated by changes in plant volatiles of the shared host plant. Changes in the plant-volatile blend induced by root-feeding insects may alert the aboveground parasitoids about the presence of the root herbivores on the host plant, which has potentially negative consequences for offspring fitness of the parasitoid. We conclude that understanding the ecology and evolution of above-belowground interactions requires an in-depth understanding of behavioural choices and preferences of the key players in the community. Further studies conducted in the field under more heterogeneous conditions and over longer time frames are needed in order to identify the above and belowground processes that maintain community structure and stability.

Acknowledgements

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Chapter 5

Foraging efficiency of a parasitoid of a leaf herbivore is influenced by root herbivory on neighbouring plants

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SUMMARY

1. Root-feeding insects can influence foliar quality of the host plant, which can affect the development and behaviour of leaf herbivores and parasitoids. Thus far, such interactions have been reported in situations where root and leaf associated organisms share a host plant. We tested whether root herbivory influences searching behaviour of an aboveground parasitoid when the foliar-feeding host of the parasitoid and the root herbivore are feeding on different plants.

2. We manipulated the proportion of 25 plants (ranging form 0 to 1) exposed to root herbivory by *Delia radicum* (neighbouring-plants). Five additional plants

were infested aboveground with *Pieris brassicae* larvae (host-infested plants) and were placed in-between the neighbouring plants. We then released females of the parasitoid *Cotesia glomerata* which attacks *P. brassicae* and studied foraging efficiency of the parasitoid.

3. Overall, parasitoids located more host-infested plants during the maximum allowed searching time, and found their hosts about three times faster when neighbouring plants were exposed to root herbivory, than when neighbouring plants were not infested with *D. radicum*. Similar results were found when the host-infested plants were also exposed to root herbivory.

4. Our results show that the interaction between an aboveground foliar-feeding insect and its parasitoid can be influenced by the presence of non-host herbivores feeding on the roots of neighbouring conspecific plants.

INTRODUCTION

Feeding by herbivorous insects often triggers the emission of specific blends of plant volatiles that attract predators and parasitoids of the herbivores, which will potentially reduce herbivore damage and thus increase plant fitness (Turlings et al. 1990). Consequently, host-herbivore induced plant volatiles are important host-location cues for parasitic insects (De Moraes et al. 1998). Female parasitoids of herbivorous insects spend most of their life searching for the most profitable hosts in which to lay their eggs, and odours coming from the host itself are often more difficult to detect from a distance than plant derived volatiles (Vet and Dicke 1992). Since the discovery of herbivore-induced plant volatiles, a central theme in this area has been the study of potential mutualistic associations between plants and insect parasitoids (Vet and Dicke 1992).

In terrestrial ecosystems, insect parasitoids also interact with other species of non-host herbivores feeding on the same host plant. Recent research has shown that the volatile composition of plants infested with multiple herbivore

species may differ from plants infested with a single herbivore species (Shiojiri et al. 2001, 2002). Attack from multiple species of herbivores may result in chemical interference and can make host-containing plants either less or more attractive to the foraging parasitoids (Shiojiri et al. 2001, 2002). The non-host herbivores can cause parasitoids to waste time in searching for, and in the handling of non-hosts, weakening the consumer (parasitoid)-resource (host-herbivore) interaction. Interference by non-host herbivores has been theoretically proposed to play an important role in the stability of food webs by generating the presence of spatio-temporal refuges (McCann et al. 1998, Vos et al. 2001, Gols et al. 2005). Under conditions where selection from top-down (natural enemy) pressures is severe, refuges prevent the over-exploitation of herbivore populations allowing for their persistence in space and time (Begon et al. 1996).

Recently, White and Andow (2006) reported that root-feeding insects can also influence host searching behaviour by a specialised parasitoid of an aboveground herbivore. The presence of root-feeding insects reduced parasitism rates of the aboveground herbivore, via changes in plant height and density (White and Andow 2006). Moreover, root-feeding insects can induce changes in the plant volatile blend and this can deter aboveground parasitoids foraging for foliar-feeding hosts on the shared host plant (Soler et al. 2007).

Thus far, interactions between spatially separated organisms have been reported in situations where root and leaf associated organisms share a host plant (Bezemer et al. 2003, Guerrieri et al. 2004, Soler et al. 2005). Here we present novel evidence that the interaction between the foliar-feeding insect *Pieris brassicae* L. (Lepidoptera: Pieridae) and its endoparasitoid *Cotesia glomerata* L. (Hymenoptera: Ichneumonidae) can be influenced by root herbivory by larvae of the cabbage root fly *Delia radicum* L. (Diptera: Anthomyiidae) 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 plants that were infested aboveground with their hosts was recorded. The experiment was

repeated with an identical set-up but with host-infested plants that were also exposed to root herbivores.

MATERIALS AND METHODS

Studied system

The study was based on a naturally occurring multitrophic system in Western Europe. The black mustard, *Brassica nigra* L. (Brassicaceae), is a widely distributed annual wild crucifer that is common along rivers and in disturbed areas in The Netherlands. Generally, this cruciferous plant grows in extended patches and can become a dominant species in early successional habitats (Schaminée et al. 1998). It is attacked by several leaf-feeding herbivores, including larvae of the large cabbage white butterfly, *P. brassicae* (Harvey et al. 2003). *Cotesia glomerata* is a gregarious endoparasitoid that attacks young larvae of several species of pierid butterflies, with *P. brassicae* being its preferred host in much of the Paleractic (Feltwell 1982). Mustard plants are also attacked by specialised root-feeding herbivores, including larvae of the cabbage root fly, *D. radicum* (Coaker and Finch 1971). The root fly is known to attack many species of crucifers and prefers to oviposit in plants that were previously damaged by conspecific larvae (Baur et al. 1996).

Plants and insects

Pieris brassicae larvae were obtained from an insect culture maintained at the Laboratory of Entomology of Wageningen University, The Netherlands, where it is reared on *Brassica oleracea* L. cv Cyrus plants. *Cotesia glomerata* and *D. radicum* were obtained from cultures maintained at the Netherlands Institute of Ecology, Heteren, The Netherlands. The root herbivore was cultured on roots of *Brassica napus* L. plants. *Brassica nigra* seeds were collected from a single population growing just to the Northwest of Wageningen, The Netherlands.

Seeds were surface sterilised and germinated on a bed of glass-pearls (pearls of 1 mm diameter). One week after germination seedlings were transplanted into 1.2 litre pots filled with sterilised potting compost. The plants were grown in a greenhouse, at 22 ± 1 $^\mathrm{o}$C (day) and 16 ± 1 $^\mathrm{o}$C (night), 70% RH and 16:8 h light:dark photoperiod. Natural daylight was supplemented by metal-halide lamps (225 µmol s^{-1} m^{-2} PAR). Plants were watered daily and only non-flowering plants were used in the experiments.

Experimental set-up

The experiment was conducted in a greenhouse compartment, at 22 ± 1 $^\mathrm{o}$C and 70% RH, inside a specially constructed tent (3 m x 2 m x 3 m). The tent was made of transparent net to allow for illumination and air-circulation. Moreover, the greenhouse compartment was continuously ventilated to improve air-circulation. To avoid interference between plant-odours from different replicates and treatments, the air inside the tent was ventilated between replicates using a fan. Natural daylight was supplemented by metal-halide lamps (225 µmol s^{-1} m^{-2} PAR).

To test the effects of root herbivory on neighbouring plants on the foraging behaviour of the parasitoid, we compared the time that the parasitoid spent to locate five host-infested plants (*B. nigra* + *P. brassicae*) placed in four habitats consisting of 25 conspecific plants with different proportions of plants exposed to root herbivory (0, 0.2, 0.6 or 1) and without *P. brassicae*. Inside the tent the 30 *B. nigra* plants were placed in five rows of six plants. The distance between adjacent plants was 30 cm. On the five 'host-infested plants', broods of ten first instar *P. brassicae* larvae were placed on the youngest fully developed leaf one day prior to each test. The larvae were enclosed in a cylindrical clip-on cage (5.5 cm diameter) to ensure that they stayed on the plant. Clip-on cages were removed before starting each test. The other 25 plants (habitat plants) were not infested with *P. brassicae* (Fig. 1). The position of the host-infested and habitat plants was determined randomly and kept constant in all replicates (Fig.

1). The experiment was performed with host-infested plants without root herbivory and was subsequently repeated using host-infested plants that were also exposed to *D. radicum*. Plants exposed to root herbivory, either host-infested or habitat plants, had been exposed to eight L3 *D. radicum* larvae for a period of six days prior to their use in the tent. Previous results have indicated that in this system this is sufficient for volatiles to be emitted aboveground (Soler et al. 2007). Each 'habitat' (0, 0.2, 0.6, 1) was replicated four times. In habitat '0' fewer host-patches were located by *C. glomerata* compared to other habitats (see Results). Therefore, for the first set of tests with host-infested plants that were not exposed to root herbivory, five further replicates were added to this treatment (Table 2). In each replicate new plants were used. Plants of similar height and shape were used to avoid the influence of any potential confounding effects of plant characteristics on parasitoid behaviour. We previously reported that in our experimental conditions the density of eight *D. radicum* larvae per plant does not affect aboveground biomass production (Soler et al. 2005).
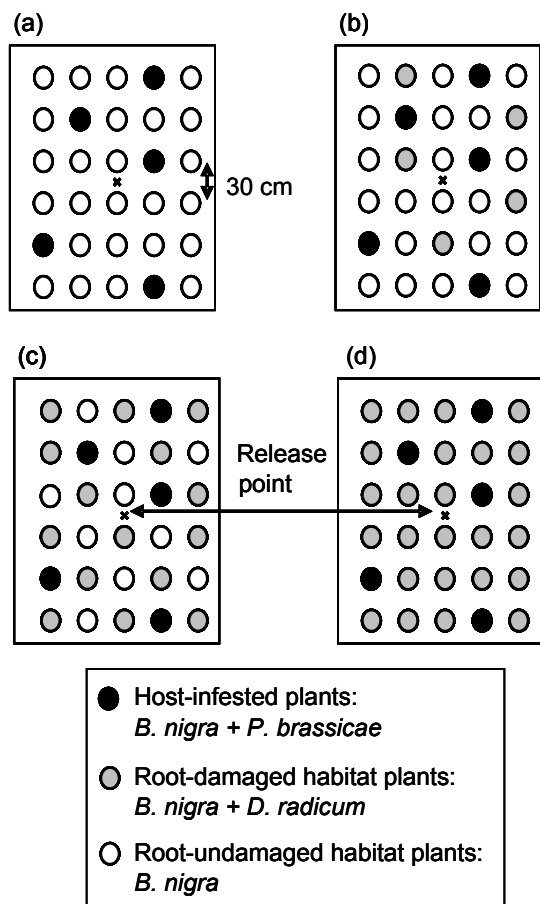
Figure 1. Scheme of the experimental set-up. Distribution of the five host-infested plants which served as parasitoid target-plants, and the habitat plants (without hosts). The four habitats consisted of 25 neighbouring *B. nigra* plants of which a proportion of (a) 0, (b) 0.2, (c) 0.6 or (d) 1 was exposed to root herbivory by *D. radicum*. Five parasitoid females were released in the centre of the habitat, and were observed until all five host-infested plants were located, or for a maximum of 70 minutes. The experiment was repeated using host-infested plants that were also exposed to root herbivory.

Each replicate started by releasing and observing, simultaneously, five parasitoid females in the centre of the tent. We recorded the time (in minutes) that it took for a parasitoid, independent which of the five, to alight for the first time on a leaf of each of the five host-infested plants. In most cases the female parasitoids landed directly onto the leaf on which the brood of hosts was present. In some occasions the wasps landed on an uninfested leaf of a host-infested plant and took a few more seconds before locating caterpillars and commencing to parasitise them. Larvae were subsequently dissected to ensure that caterpillars were indeed parasitised. Each replicate was terminated when all five host-infested plants were located, or after a period of 70 minutes (based on pilot experiments). If after this period not all the host-infested plants had been located, the number of host-infested plants that were not located was recorded. To increase the parasitoid's motivation to search for hosts, one first instar larva of *P. brassicae* was presented to each female wasp for oviposition immediately prior to each test (Vet et al. 1995). These first instar larvae had been feeding on a different host plant (*B. oleracea*) to prevent the possibility of associative learning of *C. glomerata* affecting the plant choice in the test (Vet et al. 1995).

Statistical analysis

The effect of root herbivory in the surrounding habitat (0, 0.2, 0.6 or 1) and host-infested plant status (root-undamaged or root-damaged) on the time spent by the parasitoids to find the first, second, third, fourth and fifth host-infested plant was tested using a Linear Mixed Model. In this test only host plants that had been located were included. The mixed model was fitted by restricted maximum likelihood (REML) in Genstat. Based on residual inspection the data were log-transformed to stabilise the variance. Tests for fixed effects were based on approximated F-tests and means compared by calculating the least significant difference (LSD) based on the standard errors of the differences estimated by REML. The effect of the different environments on the number of host-infested

plants found during the maximum allowed searching time was determined with a Chi-square test.

RESULTS

The time to locate host-infested plants differed significantly between habitats, and also varied along the sequence of located host-infested plants (Table 1). In habitats composed entirely of root-undamaged plants, parasitoids spent significantly more time to locate the five host-infested plants compared to habitats containing root-damaged plants (Fig. 1). On average parasitoids took 42 minutes to locate all host-infested plants in habitats without plants with root herbivory, and 10-17 minutes in habitats with different proportions of habitat plants with root herbivory (Fig. 2). Host-location time was similar in the three habitats where root-damaged habitat plants were present at different densities. There was no effect of habitat on host-location for the first and second host-infested plant, but the differences between the habitats with and without root herbivore-infested habitat plants became apparent for the subsequent host-infested plants. Whether host-infested plants were exposed to root herbivory or not did not significantly influence the response of the parasitoid (Table 1, Fig. 2).

Table 1. Approximate F-test (degrees of freedom, F and P values) for the fixed effects from REML analysis of the effect of habitat (composed of 25 *B. nigra* plants of which a proportion of 0, 0.2, 0.6, or 1 was exposed to root herbivory by *D. radicum*), and status of the host-infested plants (with or without root herbivory), on the average time spent by the parasitoid females to find the 1st, 2nd, 3rd, 4th, 5th host-infested plant.

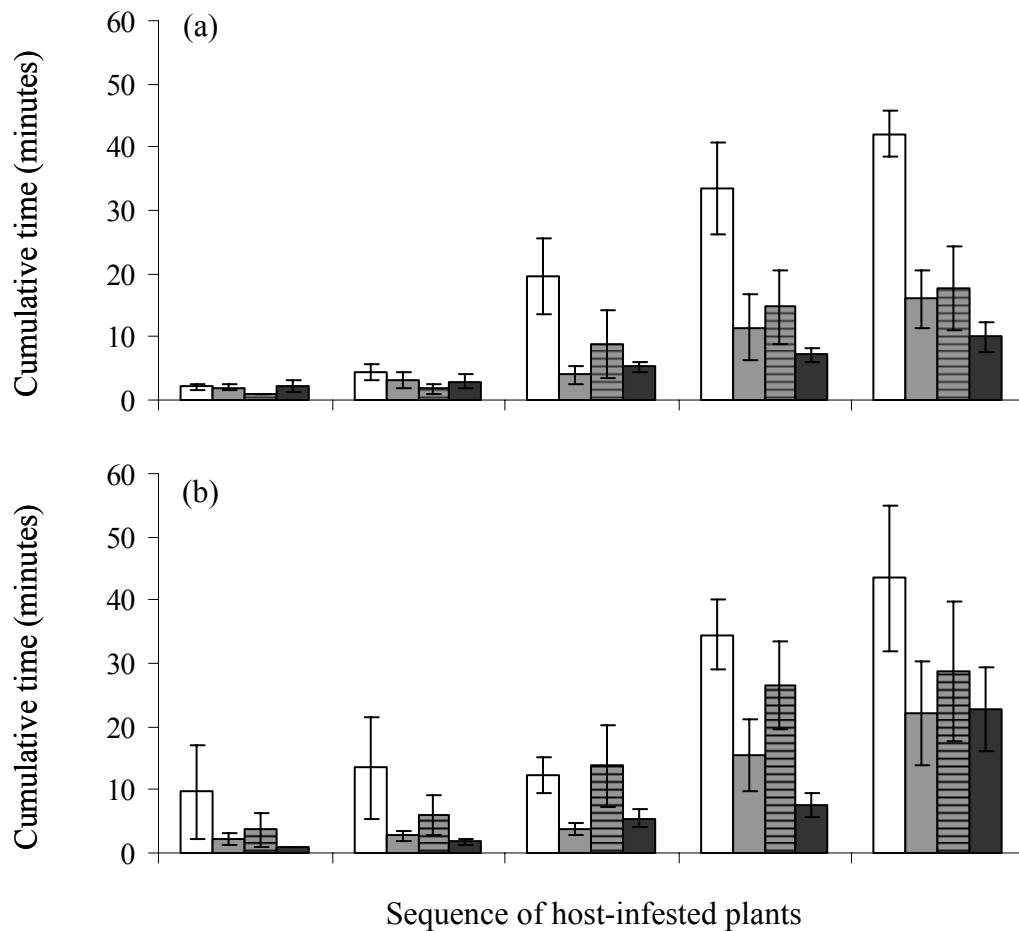| Factors		First		Second		Third		Fourth		Fifth	
	df	F	P	F	P	F	P	F	P	F	P
Habitat (H)	3	0.86	0.46	2.07	0.10	3.61	0.01	5.13	0.002	4.86	0.002
Status (S)	1	1.01	0.31	1.54	0.21	0.02	0.89	1.51	0.21	2.58	0.10
H * S	3	1.45	0.22	1.36	0.25	0.11	0.95	0.35	0.79	0.37	0.77

Figure 2. Mean (± SE) cumulative time (minutes) spent by female parasitoids to find five host-infested plants in four habitats composed of 25 B. *nigra* plants of which a proportion of 0 (white bars), 0.2 (grey bars), 0.6 (striped bars), or 1 (black bars) of the plants were exposed to root herbivory by *D. radicum*. Although there were no significant effects of host-infested plant 'status', data are shown separately for (a) host-infested plants without root herbivory and (b) host-infested plants exposed to root herbivory.

The number of host-infested plants that was found during the allowed searching time also differed significantly between habitats ($\chi2$ = 4.07; df = 1; P = 0.043; Table 2). Fewer host-infested plants were located in habitats composed entirely of plants without root herbivory, compared to habitats with different proportions of root-damaged plants ($\chi2$ = 4.94; df = 1; P = 0.026). The number of host-infested plants found, did not differ between the three habitats where habitat plants with root herbivory were present at different densities ($\chi2$ = 0.00; df = 1; P = 1.00).

Table 2. Successful location of host-infested plants during the maximum allowed searching time of 70 minutes, in the different habitats (composed of 25 *B. nigra* plants of which a proportion of 0, 0.2, 0.6, or 1 was exposed to root herbivory by *D. radicum*). Shown are mean (± SE) of the proportion of replicates in which three, four, or five host-infested plants were located. In all replicates at least two host-infested plants were located. Number of replicates is also presented. Host-infested plants were with or without root herbivory.

Host-infested plant	N	Habitat	Number of host-infested plants located		
			3	4	5
No root herbivory					
	9	0	1.0 ± 0	1.0 ± 0	0.78 ± 0.15
	4	0.2	1.0 ± 0	1.0 ± 0	1.0 ± 0
	4	0.6	1.0 ± 0	1.0 ± 0	1.0 ± 0
	4	1	1.0 ± 0	1.0 ± 0	1.0 ± 0
Root herbivory					
	4	0	0.75 ± 0.25	0.50 ± 0.29	0.50 ± 0.29
	4	0.2	1.0 ± 0	1.0 ± 0	1.0 ± 0
	4	0.6	1.0 ± 0	1.0 ± 0	0.75 ± 0.25
	4	1	1.0 ± 0	1.0 ± 0	1.0 ± 0

DISCUSSION

Studies linking interactions between aboveground and belowground insects have traditionally been focused on organisms intimately associated with the same plant (Gange and Brown 1989, Masters and Brown 1992, Bezemer et al. 2003, Bezemer and Van Dam 2005, Soler et al. 2005). These studies have shown that the development and behaviour of insect herbivores and their natural enemies can be influenced by root herbivores that share the host plant (Gange et al. 2003, Guerrieri et al. 2004, Bezemer et al. 2005, Soler et al. 2005). A recent study

examined preference behaviour in *C. glomerata* for hosts feeding on plants that were also exposed to the root herbivore *D. radicum* compared with hosts feeding on plants without root herbivory. In that study, *C. glomerata* exhibited a clear preference to search for and oviposit in hosts feeding on plants without root herbivory (Soler et al. 2007). This behaviour may be an adaptive response that enables the parasitoid mother to avoid high levels of phytotoxins in the foliage of plants induced by root herbivory, which would otherwise negatively affect the performance of her progeny (Soler et al. 2005). In the present study we show that root herbivores can also influence aboveground host-parasitoid interactions via changes in the 'attractiveness' of surrounding conspecific plants.

Complexes of herbivore species on a single plant can diminish the searching efficiency of foraging parasitoids, either by making the volatile blend of the host-infested plants less reliable for searching parasitoids or by causing parasitoids to waste time searching on the non-host larvae (McCann et al. 1998, Vos et al. 2001). In the present study 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. We previously reported that *B. nigra* plants exposed to root herbivory emit a volatile blend characterised by high levels of specific sulphur volatile compounds, and it appears that these volatile cues are used by the parasitoid females to identify and avoid plants with root herbivory (Soler et al. 2007). We propose that the characteristic volatile blend emitted by habitat plants exposed to root herbivory that surrounded the host-infested plants may have provided a more contrasting background than the blend emitted by habitat plants without root herbivory, 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 searching on or near plants without hosts. In our study system plants without hosts, but exposed to root herbivory, repel searching parasitoids (Soler et al. 2007), 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. A shortcoming of the present study is that we did not record the time spent by parasitoids on the habitat plants, and more work is required in which parasitoid behaviour is recorded continuously on plants with and without hosts in complex habitats.

Habitat characteristics can play profoundly important roles in the dynamics of parasitoid-host interactions (Ellner et al. 2001, Vos et al. 2001, Gols et al. 2005). Background odours and contrasts in odours, for example, are important cues in the foraging behaviour of parasitoids, even when the parasitoids respond to a specific single volatile compound in the blend (Mumm and Hilker 2005). The results of the current study suggest that root herbivory on surrounding plants provided more contrast between the host-infested plants and the surrounding plants than undamaged plants. Whether host-infested plants were exposed to root herbivory or not did not influence the behavioural pattern. This suggests that the attractiveness of host-infested plants was similar, independent of whether the plants were also exposed to root herbivory, and that the contrast between host-infested plants and root-damaged habitat plants was strong independent of root herbivory on the host-infested plant. A previous study with the same system showed that, independent of whether plants are also exposed to root herbivory, volatile blends of *B*. *nigra* plants exposed to foliar herbivory are distinctly different from plants only exposed to root herbivory or undamaged plants (Soler et al. 2007). It is important to stress that in the current experiment parasitoids could not choose between hosts on plants with and without root herbivory. When hosts were available on plants also exposed to *D*. *radicum*, the attraction to host-related cues must have been stronger than the repellent effects of cues related to root herbivory on the host plant.

Whether the surrounding plants were exposed to root herbivory or not did not affect location of the first and second host-infested plant. Alternatively, this did strongly affect location of sequential plants by the parasitoids. Obviously, the probability of randomly locating host-infested plants diminishes with each

located plant and this could explain these results. It is also possible that, given more time and experience, parasitoids would be better able to assess the quality of their habitat, and that they would then adjust their behaviour accordingly. It is well established that the host-finding success of parasitoids, including *C. glomerata*, improves with time through learning (reviewed by Vet et al. 1995). It remains unknown whether and how learning processes in insect parasitoids can be influenced by interactions that occur in the rhizosphere.

An increasing body of literature has emphasised that soil dwelling organisms can influence aboveground trophic interactions when the spatially separated consumers share the same plant (e.g. Masters and Brown 1992, Van der Putten et al. 2001, Wardle et al. 2004, Bezemer and Van Dam 2005). This study has shown that the effects of belowground processes can also affect the behaviour of insects indirectly via the surrounding environment. Consequently, the potential effects of soil organisms on aboveground systems may still be much greater than has been proposed thus far.

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Chapter 6

Plant preference of foliar herbivores is influenced by root herbivores: a field study

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SUMMARY

There is a strong selection pressure for herbivorous insects to feed on plants with high nutritional quality to optimise their performance and to oviposit on plants that will maximise the performance of their off-spring. Root-feeding insects can influence the biomass and quality of the plant shoot, which, in turn, can affect the performance of the foliar insect herbivores sharing the host plant aboveground. In the field we studied whether root-feeding by larvae of the cabbage root fly, *Delia radicum* on the wild crucifer plant *B. nigra* influences plant preference and oviposition behaviour of naturally occurring foliar-feeding insect herbivores. In an ungrased grassland, we set-up eight plots in four blocks with 10 root-damaged and 10 root-undamaged potted *B. nigra* plants in each plot. To study the effect of the spatial distribution of the root herbivore, in each block in one plot the root-

damaged and the root-undamaged plants were alternated while in the other plot the plants were grouped in clusters of root-damaged and root-undamaged plants. Overall, specialised foliar-feeders (flea beetles, aphids and butterflies) preferred to lay eggs and feed on root-undamaged *B. nigra* plants over plants exposed to *D. radicum*. On the contrary, the generalist insect aphid species (*Myzus persicae*) did not discriminate between root-damaged and root-undamaged plants. Remarkably, the specialised aphid species *B. brassicae* strongly preferred to feed on root-undamaged plants, but only in clustered plots. The implication of the presence of and distribution over plants of subterraneous insects, whose presence is frequently unnoticed, for plant acceptance of foliar-feeding insects is discussed.

INTRODUCTION

Host plant selection by foliar insects is often divided into 'host plant finding and 'host plant acceptance" (Schoonhoven 1972). Depending on the species, foliar feeders can identify from a distance a host plant exploiting odours and visual cues of the plant (Finch and Collier 2000). Other species posses mainly post-alighting recognition and therefore find their host plants predominately based on contact-sensory information of the plant surface (Klijnstra and Schoonhoven 1987, Bernays 1991, Renwick and Chew 1994). For most foliar-feeding insects, host plant acceptance is governed by contact chemoreception of secondary plant compounds (Schoonhoven et al. 2005). Most specialised insect herbivores exclusively feed on plants that produce a specific group of secondary plant compounds (Bernays and Graham 1988). Specialist insects normally deal with high levels of specific secondary plant compounds in their diet although their development can be negatively influenced when feeding on plant species or genotypes containing excessively high levels of phytotoxins (Adler et al. 1995, Schoonhoven et al. 2005). These phytotoxins usually act as deterrents to non-

adapted generalist insect herbivores (Schoonhoven et al. 2005).

The majority of studies on host plant selection have focussed on interactions between foliar herbivores and cruciferous plants in an aboveground context (Finch and Collier 2000). Cruciferous vegetables and oil seed crops are of high economic importance; their chemistry is well understood and they support insect species of a wide range of orders (Finch 1993). Cruciferous plants contain glucosinolates, and the negative impact of these secondary plant compounds on generalist insect herbivores as well as their use as kairomones by specialised feeders that exclusively feed on plants that produce them has been studied extensively (Siemens and Mitchel-Olds 1998). Glucosinolates are toxic and act as feeding deterrent to non-adapted herbivores (Coleman et al. 1996, Hopkins et al. 1998), but normally act as feeding and oviposition stimulants for specialised crucifer-feeders (Bartlet et al. 1994). However, recent studies have shown that root-feeding insects on cruciferous plants can significantly increase the levels of glucosinolates in the foliage of the plant, compromising the development of specialised crucifer-feeding insects such as *Pieris brasssicae* (Soler et al. 2005, 2007, Van Dam et al. 2005). Root feeders can also induce changes in the volatile blend of the plant aboveground (Rasmann et al. 2005, Soler et al. 2007), that in turn, influences the behaviour of carnivores of foliar-feeding insects (Bezemer et al. 2005, Guerrieri et al. 2004, Poveda et al. 2005, Soler et al. 2007). Therefore, root feeders can change both the quality of the leaves and the volatile blend of the plant aboveground. In this study we investigate in the field whether root-feeding damage by *Delia radicum* larvae influence the plant preference of naturally colonizing leaf herbivores associated with the wild cruciferous plant black mustard (*Brassica nigra*). We tested the hypothesis that foliar herbivores will prefer to feed and oviposit on root-undamaged plants, wich in turn will maximise their performance and the performance of their off-spring.

Delia radicum prefers to oviposit in plants that were previously damaged by conspecific larvae. This often causes a clustered distribution of root infested

plants within a *B. nigra* patch (Baur et al. 1996). To determine whether the spatial distribution of the root herbivore influences host plant selection of aboveground insects, we set-up the experiment with field plots containing plants that had either a clustered or a regular distribution of the root-infested and the root-uninfested plants. We tested the hypothesis that root-damaged plants placed in the field forming clusters will be less colonised by foliar herbivores than a cluster of plants combining root-damaged and root-undamaged plants interspersed. We expect that plants distributed in clusters would emit a stronger odour plume created by the clustered stand of plants that will warn the foliar herbivores that posses pre-alighting plant recognition about the presence of root-feeding insects belowground.

MATERIALS AND METHODS

Studied system

This study is based on interactions between naturally occurring root and shoot insect herbivores feeding on cruciferous plants. The black mustard, *Brassica nigra* L. (Brassicaceae), is a widely distributed annual wild crucifer that is common along rivers and disturbed areas in The Netherlands. Generally, this cruciferous plant grows in extended patches being the dominant species in early succession habitats (Schaminée et al. 1998). Like other members of the *Brassicaceae*, *B. nigra* possesses inducible direct and indirect defences via the production of glucosinolates and their breakdown products. These make *B. nigra* an excellent model plant for studying plant-mediated multitrophic interactions. Black mustard plants are attacked by specialised root-feeding herbivores, including the cabbage root fly, *Delia radicum* L. (Diptera: Anthomyiidae) (Coaker and Finch 1971). Aboveground, *B. nigra* is attacked by a complex of crucifer leaf-herbivore insect species belonging to the orders Hemiptera, Lepidoptera and Coleoptera (Nielsen 1977, 1978).

Plants and insect cultures

Delia radicum larvae were obtained from insect cultures maintained at the Laboratory of Ecobiology of Insect Parasitoids, of Rennes University, France, cultured on roots of *Brassica napus* L. plants. *Brassica nigra* seeds were collected from a single population in Wageningen, The Netherlands. Seeds were surface sterilised and germinated on a bed of glass-pearls (pearls of 1 mm diameter). One week after germination seedlings were transplanted into 1.2 l pots. The plants were grown in a greenhouse, at 22 ± 1°C (day) and 16 ± 1°C (night), 70% RH and 16:8 h light:dark photoperiod. Natural daylight was supplemented by metal-halide lamps (225 µmol s^{-1} m^{-2} PAR). Plants were watered daily and only non-flowering plants were used in the experiments.

Experimental set-up

In June-August 2006 we set-up a field experiment to evaluate the effect of root-feeding by *D. radicum on B. nigra* plants on the host plant preference of foliar herbivore insects. Within a flat area of about 1.5 Ha, we placed a total of 160 individually potted *B. nigra* plants. Half of the plants were root-damaged (by *D. radicum*) and half were root-undamaged and served as control. The plants were placed in the field in four blocks. In each block there were two plots with 10 root-damaged plants and 10 root-undamaged plants. One of the two plots had a regular distribution of the two types of plants, with root-damaged and root-undamaged plants alternated. In the other plot, root-damaged and root-undamaged plants were both placed in four clusters of five plants (Fig. 1). For both distributions, the distance between adjacent plants was 1.2 m and the distance between the plots was 11 m. The experiment was repeated three times during the summer of 2006 (June, July, and August). For each replicate new plants were used.
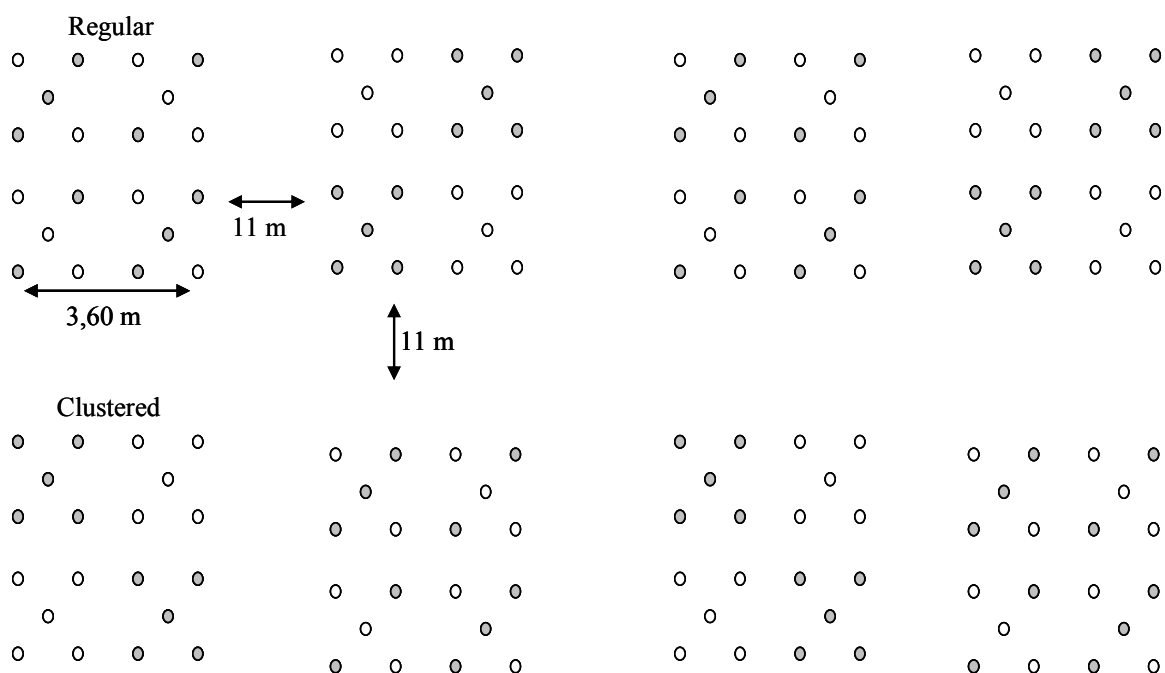
Regular

Figure 1. Scheme of the experimental set up. On each date, June, July and August, 160 plant were placed in the field in four blocks as described in the figure. Distance between plots was 11 metres. White circles represent root-undamaged plants and grey circles represent plants damaged by *D. radicum*. In each block, in one plot the plants were distributed in clusters while in the other plot they were distributed regularly.

Prior to their use in the experiment, all plants were grown under controlled conditions in a greenhouse in 1.2 l pots filled with sterilised potting soil. Holes in the bottom of each pot, to ensure proper drainage, were covered by a gauze. Three to four weeks after transplanting, six last instar *D. radicum* larvae were introduced into pots assigned to the root herbivory treatment. The larvae were carefully placed with a small brush next to the main stem of the plant and observed until they successfully crawled into the soil. After introduction of the root herbivore larvae, the soil surface of each pot was carefully covered by a gauze that was fixed onto the pot with a rubber band, to prevent additional soil infestation. Seven days after introduction of the *D. radicum* larvae, the plants were placed in the field. Previous work has shown that in *B. nigra*, shoot-glucosinolate levels significantly increase approximately seven days after initiation of root herbivory (Van Dam and Raaijmakers 2006).

Each (potted) plant was placed in a hole previously made in the soil at fixed positions, so that the soil surface level of the potted plants was identical to the level of the soil surface of the field. In the greenhouse and in the field all plants were watered daily. For each plot, plants of similar height and shape were selected to minimise any potential effects of plant characteristics on host plant selection behaviour. The experiment was performed in June, July and August, and each time the plants were kept in the field for a period of three days. During this period, every day during early morning and late afternoon, all plants were checked and all insects and insect eggs present on the foliage of each plant were collected, and stored for subsequent identification. Immediately after each experiment, ten randomly selected root-damaged experimental plants were checked to ensure that *D. radicum* larvae were present and actively feeding. More than 80 percent of the larvae that were introduced were recovered during each experiment-date.

Statistical analysis

The effects of root herbivory (with or without root herbivory) and spatial distribution of plants (clustered or regular) and their interaction on the plant preference of naturally colonizing foliar herbivore insects associated to *B. nigra* plants was tested using a Generalised Linear Mixed Model (Poisson distribution and log link function). The mixed model was fitted by restricted maximum likelihood (REML) in Genstat.

RESULTS

A total of 2581 insects and insect eggs were collected from the experimental *B. nigra* plants. These insects were classified into adults and nymphs of the flea beetle species *Phyllotreta* spp. (Coleoptera: Alticinae), aphids of the species *Brevicoryne brassicae* (Hemiptera: Aphidae) and the 'green peach aphid' *Myzus*

persicae (Hemiptera: Aphidae), and eggs of the 'small white butterfly' *Pieris rapae* (Lepidoptera: Pieridae). The differentiation at species level of *Phyllotreta undulata* and *Phyllotreta diadema* could not be determined, and therefore both flea beetles were grouped together. All these species are commonly observed on cruciferous plants and are specialised herbivores except for the aphid *M. persicae* that is a generalist. We also collected eggs and adults of the 'green lacewing'generalist predator *Chrysoperla* carnea (Neuroptera: Chrysopidae) which was frequently observed on the foliage of the experimental *B. nigra* plants. More than 90% of the insects were collected during the second sampling date (July; Fig. 2). Subsequent analyses were therefore carried out for this collection date only.
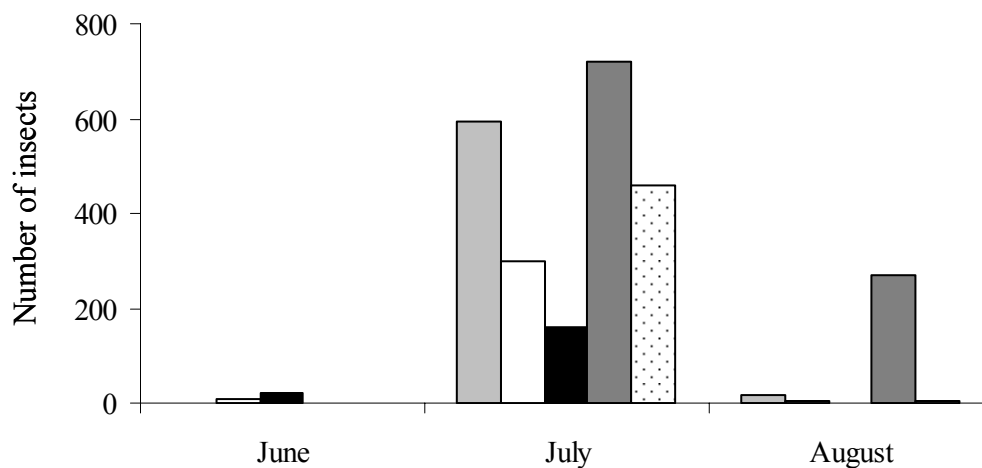
Figure 2. Cumulative number of adults and nymphs of *Phyllotreta* spp. (light grey bars), *B. brassicae* (white bars) and *M. persicae*, (black bars) and eggs of *P. rapae* (dark grey bars) and *C. carnea* (dotted bars), on the foliage of *B. nigra* plants during the three months in which the experiment was repeated.

Root herbivory and the distribution of the root-damaged and root-undamaged plants both affected insect abundance. However, the effects differed strongly between insect species (Table 1). *Phyllotreta* spp. significantly preferred to feed on root-undamaged over root-damaged plants, independent of the distribution of root-damaged plants (Fig. 3). *B. brassicae* significantly preferred to feed on root-undamaged plants, exclusively when the root-damaged and root-

undamaged plants were clustered. The plant preference of *M. persicae* was not influenced by the presence or distribution of root-feeding insects (Table 1, Fig. 3). *P. rapae* laid more eggs on root-undamaged than on root-damaged plants. Independent of root herbivory, fewer *P. rapae* eggs were observed in plots with clustered distributions than in regular plots (Fig. 3). Host preference of the insect predator *Chrysoperla carnea* was not significantly influenced by the presence of root-feeding insects. However, fewer eggs were found in clustered than in regular plots (Table 1, Fig. 4).

Table 1. Approximate F-test (F and P values) for the fixed effects from GLMM analysis of the effect of root herbivory by *D. radicum* (presence or absence), and distribution (regular or clustered) of the root-damaged and root-undamaged plants, on the number of adults and nymphs of *Phyllotreta* spp., *B. brassicae* and *M. persicae*, and eggs of *P. rapae* and *C. carnea*, on the foliage of *B. nigra* plants.

Factors	*Phyllotreta* sp.		*B. brassicae*		*M. persicae*		*P. rapae*		*C. carnea*	
	F	P	F	P	F	P	F	P	F	P
Root herbivores (RH)	37.82	<0.001	11.40	<0.001	2.90	0.09	6.69	0.01	3.57	0.06
Distribution (D)	0.17	0.68	0.40	0.52	0.40	0.52	6.52	0.01	21.94	<0.001
RH * D	0.62	0.43	10.27	0.001	0.32	0.57	3.40	0.07	0.09	0.75
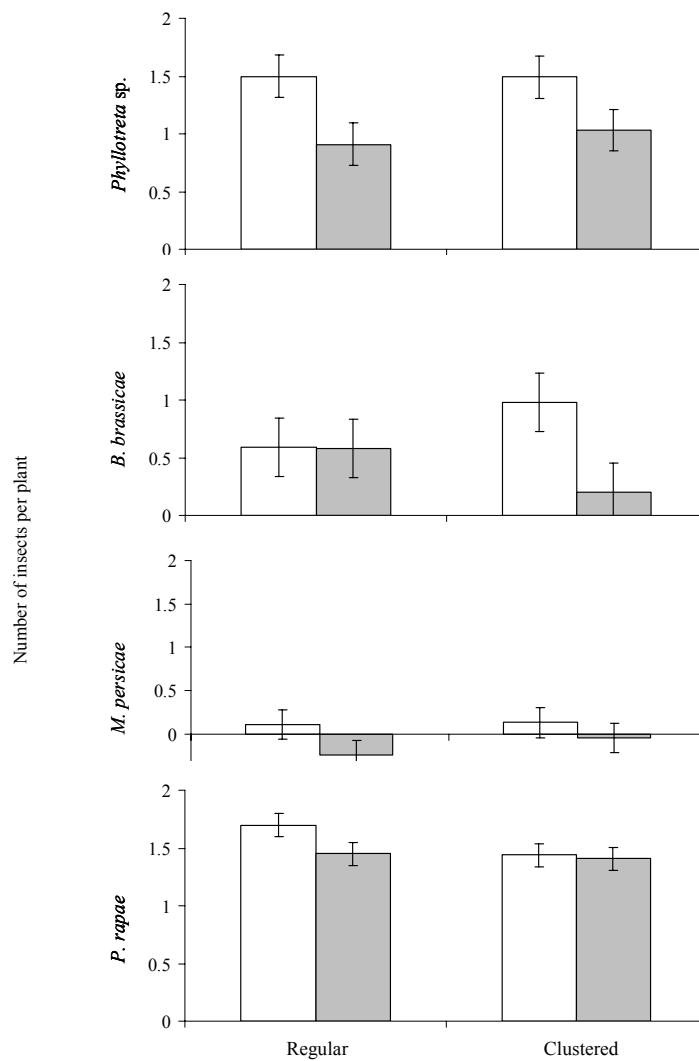
Figure 3. GLMM estimates (± SE of the difference) of the number of herbivore insects feeding per plant, in the foliage of *B. nigra* plants. White bars represent root-undamaged plants and grey bars represent root-damaged plants. Plots had either a regular or a clustered distribution of root-damaged and root-undamaged plants. Although there were no interaction effects for most of the insect species, data is shown for both factors separately.
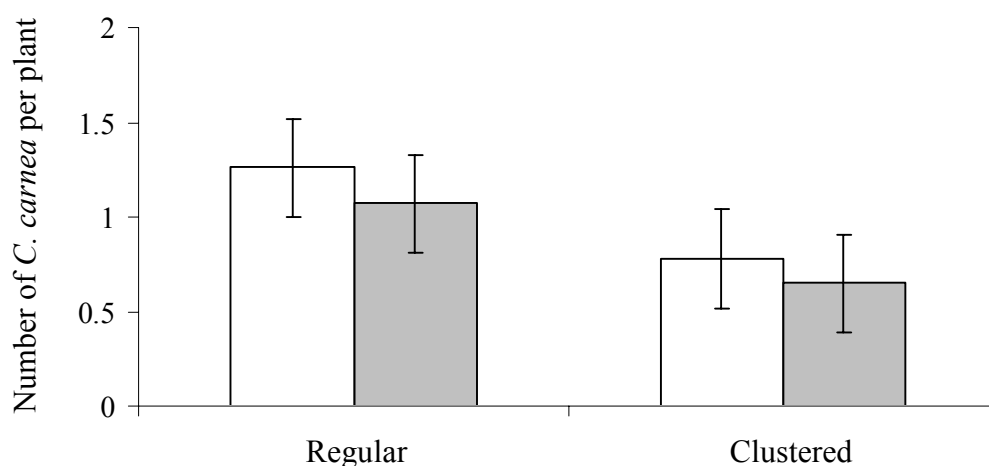
Figure 4. GLMM estimates of the number of eggs of the predator *C. carnea* per plant, in the foliage of *B. nigra* plants. White bars represent root-undamaged plants and grey bars represent root-damaged plants. Plots had either a regular or a clustered distribution of root-damaged and root-undamaged plants.

DISCUSSION

In this field study we found a general preference of the specialised crucifer foliar-feeders to feed and oviposit on plants that are not shared with root-feeding insects. The foliar feeders specialised on glucosinolates (*Phylotreta* sp., *B. brassicae* and *P. rapae*) preferred to lay eggs and feed on root-undamaged *B. nigra* plants over plants exposed to root-feeding insects belowground. The preference-performance hypothesis predicts a strong selection pressure for specialised insects to feed and oviposit on plants with high nutritional quality, so that their performance will be maximised (Jaenike 1978). In a previous study we found that the performance of a specialised aboveground crucifer-feeder, *Pieris brassicae*, was significantly reduced when the larvae feed on plants shared with the root-feeder insect *D. radicum* (Soler et al. 2005). Plants exposed to *D. radicum* showed higher levels of glucosinolates (double level of sinigrin) in the shoot than root-undamaged plants, which likely negatively affected the development of *P. brassicae* (Soler et al. 2005). It is possible that doubling levels

of glucosinolates in the shoot of the plants exposed to root herbivores may have caused the discrimination against those plants by the specialised foliar feeders in the field experiment. However, specialist insects are expected not to be negatively influenced by the presence of secondary plant compounds in the relatively few species of plants that they feed upon. Nevertheless it has been shown, for example for *Phyllotreta* beetles, that sinigrin acts as a feeding stimulant at certain levels, but that high levels are less stimulatory (Hicks and Tahvania 1974, Louda and Rodman 1983). In the contrary, the only generalist herbivore abundantly present on *B. nigra* shoots in our experiments, the phloem sucker *M. persicae*, did not discriminate between root-damaged and root-undamaged plants. This aphid species is known to feed on hundreds of host plants species, distributed over 30 different families (Weber et al. 1986). It is a well dispersed species, extremely polyphagous and therefore it is not expected to discriminate against relatively minor differences in plant quality within a plant-species population. It remains unknown if the species recorded in this field experiment were influenced by the increased levels of glucosinolates induced by root-feeding insects. Further studies are needed to determine the plant-mediated mechanisms that result in reduced plant preference by specialised foliar-feeding insects in the presence of commonly occurring root-feeding insects belowground.

It is likely that the plots with clusters of root-damaged plants emitted a stronger or more concentrated odour plume than regularly intercalated plants. Therefore, we expected that the foliar feeders that posses pre-alighting plant recognition and discriminate against root-damaged plants would show stronger avoidance in plots with a clustered distribution. However, in our experiment, the only species responding in this way was *B. brassicae*. The specialist phloem sucker, *B. brassicae*, preferred to feed on root-undamaged plants only when there were clusters of root-damaged and root-undamaged plants. The data allow us to speculate that *B. brassicae* was able to detect the stronger volatile signal emitted from the clusters of root-damaged plants, avoiding these plants and selecting clusters of root-undamaged plants. In plots with regular distributions of root-

damaged and root-undamaged plants, *B. brassicae* may not have been able to detect the diluted signals. However, aphids in general are passive travellers that disperse with the wind compared with insects with larger body size and strong wings (Compton 2002). *B. brassicae*, among most aphid species, is thought to posses post-alighting host recognition behaviour, and uses contact cues from the plants after landing on the foliage (Bukovinszky et al. 2005). More investigation is needed to disentangle why a species that is thought to have limited long-range orientation (Nottingham et al. 1991) shows such a strong avoidance for root-damaged plants aggregated in clusters in the field.

The predatory larvae of *C. carnea* feed on aphids, nymphs of beetles, small Lepidoptera larvae among other insects (Pree et al. 1989). We recorded the eggs of *C. cornea* in our experiments, and the oviposition preference of the adult females was not significantly influenced by the presence of root-feeding insect in the plants. The larvae predate mobile prey and are very active during their entire juvenile developmental time. The predatory larvae are not restricted to the plant individual they emerge on, but rather disperse and forage for preys on other plants (Pree et al. 1989). Therefore, for this species, decisions by the adult female on where to oviposit are not decisive for the success of their offspring. It is unlikely that host plant quality is a major selection pressure for this species, and this can explain why we did not observe effects of root herbivory on *C. carnea* oviposition behaviour.

Physical plant characteristics, primary and secondary plant compounds and plant volatiles have been proposed to be the central links of host plant finding and selection by foliar insects. Soil insects, among other soil dwelling organisms, exert a strong influence on these plant characteristics, strong enough to affect the performance of foliar insects feeding on the same host plant (Gange and Brown 1989, Masters et al. 1993, Masters 1995, Van der Putten 2001, Wardle 2002, Bardgett and Wardle 2003, Bezemer et al. 2003, Wardle et al. 2004). Here we present a case study where soil insects can also significantly influence the host plant preference of foliar specialised herbivores under natural

conditions, where complex interactions take place simultaneously. To our knowledge, soil dwelling organisms are not usually considered when investigating plant finding by foliar insect herbivores. However, our study shows that they might cause patchy distributions of some of their aboveground counterparts.

Integrated Pest Managements (IPM) is a common pest control strategy in contemporary agriculture. IPM uses an array of complementary methods that include the use of pest-resistant and tolerant varieties, cultural practices, biological control and push-pull strategies with the aim of maximally reducing the use of insecticides. Push-pull strategies involve the behavioural manipulation of insects to 'push' pests away from the crop (i.e. making the plants less attractive or unsuitable to the pest), while diverting it towards an alternative source (i.e. trap-plants, attracting pheromones) (push) from where the pest can be removed (Smart et al. 1994, Kahn et al. 2000, Cook et al. 2007). All insect species identified in this study are well known economically important insect pests of *Brassica* crops. From a crop protection point of view, understanding the factors that determine host plant finding, preference and distribution of these insects may contribute to further develop and refine modern IPM programs. At present integrated pest management strategies in general are based on research focused either on aboveground or belowground scenarios separately. Although the results of this study cannot be directly applied to improve IPM strategies, it suggests that considering soil organisms associated to the plants can be important since they influence the interactions between the plants and the insect pests aboveground.

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Chapter 7

Summarising Discussion

INDIRECT PLANT-INSECT INTERACTIONS CROSSING THE BARRIER BETWEEN ABOVE- AND BELOWGROUND DOMAINS

In terrestrial ecosystems, indirect interactions occur when a third species significantly 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 multitrophic interactions has traditionally focused on organisms that share a common domain: the soil or aboveground. Considering that plants develop both underground and aboveground, they could potentially mediate indirect interactions between organisms that belong to these compartments. In the last two decades it has been emphasised that, to further understand multitrophic interactions, we need to cross the barrier between the soil and aboveground domains (Van der Putten et al. 2001, Van Dam et al. 2003). Subterranean organisms that are intimately associated with the roots of terrestrial plants have been shown to influence the levels of primary and secondary compounds within the plant, and the biomass of aboveground plant parts (Bezemer and Van Dam 2005). This, in turn, can strongly influence the growth, development and survival of foliar insects feeding on the shared host plant aboveground (Gange and Brown 1989, Masters et al. 1993, Masters 1995, Masters et al. 2001, Wardle 2002, Van der Putten et al. 2001, Wurst and Jones 2003, Bezemer and Van Dam 2005).

The discovery that soil organisms, whose presence passes habitually unnoticed, can affect the functioning of plant-herbivore communities aboveground raised the question of whether and how also higher trophic levels such as carnivores could be influenced. At present, the study of interactions between spatially separated organisms is evolving from simple interactions between organisms directly associated to the plant roots and shoot (e.g. root feeders - plant - foliar herbivores) to a more complex approach involving also higher trophic levels (e.g. root feeders - plant - foliar herbivores - parasitoids). This multitrophic approach linking food webs from the soil and the aboveground domain aims to address the complexity of populations of plants, herbivores and carnivores more realistically, to identify and further understand the forces that regulate terrestrial communities.

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

In **Chapter 2** I have shown that root-feeding insects negatively affect the performance of a foliar-feeding insect, a parasitoid and its secondary hyperparasitoid, via induced changes in the levels of secondary plant compounds of the shoot of the shared host plant. **Chapter 3** illustrates that the reverse effect also takes place. Foliar-feeding insects can negatively affect the performance of a root-feeding insect and its parasitoid, via changes in the levels of secondary plant compounds in the roots of the plant. My results provide evidence that insects in the soil can strongly interact with herbivores and carnivores aboveground, and vice-versa, that foliar insects aboveground can influence the fitness of herbivores and carnivores in the soil domain. These results emphasise 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.

A number of studies have suggested that foliar herbivores may affect soil food chains in much the same way that root herbivores affect aboveground

multitrophic interactions (Van der Putten 2003, Bardgett and Wardle 2003, Bezemer and Van Dam 2005). The results of this thesis support this, since aboveground and belowground insect herbivores negatively affected each other's performance and the performance of their parasitoids. The majority of studies exploring the influence of foliar herbivores on the performance of root herbivores show that root-feeders are negatively influenced by their aboveground counterparts. Following foliar herbivory, root herbivores show slower growth rate (Masters and Brown 1992, Masters 1995), reduced survival (This thesis, Hunt-Joshi and Blossey 2005), reduced size (This thesis, Moran and Whitham 1990, Tindall and Stout 2001), and reduced population growth (Moran and Whitham 1990, Masters et al. 1993, Salt et al. 1996). On the contrary, to my knowledge, the influence of root herbivores on the performance of foliar herbivores is not uniform, with results that are significantly positive, negative or show no effect. Therefore, we can conclude that the effect of aboveground herbivores on belowground insects is more unidirectional than is the reciprocal case. It is possible that considering feeding guilds, level of specialism and life history of the aboveground insects, as well as the identity of the plant mediating the interactions, could contribute to build more clear patterns to describe the effects of root feeders on the performance of their aboveground counterparts. For that, we need comparative studies combining different plants species mediating interactions between spatially separated insects with contrasting feeding guilds, level of specialisation and life history.

In this thesis, I have shown that the density of root herbivores also influences the responses of aboveground insects. The effects of root herbivory on the aboveground trophic chain were stronger at 'low' root herbivore density (5 *Delia* larvae per plant) than at 'high' density (20 *Delia* larvae per plant). Plants exposed to 20 larvae probably experienced severe stress and were therefore unable to maintain an optimal defense system against herbivory aboveground. 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 *B. nigra* in the Netherlands, as well as in *Brassica oleracea* (Brussels sprouts) crop fields, about five to nine *D. radicum* larvae are often found in an infested plant (R. Soler, personal observation summer 2006 and 2007, K. Winkler, personal observation). 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 aboveground and belowground organisms interact via shared host plants.

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 and Stout 2001, Blossey and Hunt-Joshi 2003). 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 (This thesis, Collantes et al. 1999), but most studies report no changes in root secondary chemistry following aboveground damage (Bezemer and Van Dam 2005). However, the results of this thesis show 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 would suggest 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 and 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.

Remarkably, in my studies the aboveground parasitoid was the most affected trophic level 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 are not only manifested up in the food chain, but can be even magnified at higher trophic levels. Recently, Bezemer et al. (2005) also reported that root herbivory by nematodes, via changes in the nutritional quality of the host plant, and foliar-feeding aphids can influence the performance of the off-spring of parasitoids aboveground. 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). In the following Chapters, I ask the question whether such negative effects of root herbivores on the performance of parasitoid progeny might have consequences for the host preference and oviposition behaviour of foraging parasitoids.

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 off-spring (Stephens and Krebs 1986). Similarly, within a host species, parasitoid females are expected to select the most profitable individuals (Godfray 1994, Brodeur et al. 1998). **In Chapter 4** I observed that parasitoid females clearly prefer searching for hosts on root-undamaged over root-damaged plants. Bearing in mind that I previously observed that the progeny of the parasitoid grows and develops poorly on root-damaged plants, this behaviour will enhance the performance of the parasitoid's

off-spring. Overall, this Chapter provides evidence that root-damaged plants convey chemical information that parasitoids can use to detect the presence of root herbivores, and avoid root-damaged plants.

This strong innate preference decreased when a clutch of hosts was placed on the shoot of the root-damaged and root-undamaged plants. However, in a semi-field experiment where I allowed parasitoids to forage freely during two hours on a group of root-damaged and root-undamaged host-infested plants, most of the hosts that were parasitised were located on root-undamaged plants. In Chapter 4 I suggest that this discrepancy between the results probably was due to volatile interference through the mixing of non-contrasting volatile blends of root-infested and root-uninfested plants with hosts in the confined flight-cage where the innate preference was tested. Alternatively, it is possible that in the semi-field experiment, given that *C. glomerata* females were allowed more time to search, female parasitoids were better able to assess the quality of their habitat, and therefore adjusted their behaviour accordingly after gaining experience. Parasitoid foraging behaviour can strongly change with experience (Lewis and Takasu 1990, Turlings et al. 1990). However, the role of parasitoid learning in dealing with natural variation in plant (and host) quality and plant volatiles induced by root herbivory remains largely unstudied. More research is needed to disentangle these complex interactions mediated via changes in the plant volatile blend triggered by belowground herbivores.

Contrary to my results, Poveda et al. (2005), using a related plant species (wild mustard, *Sinapis arvensis*) but with different aboveground 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 off-spring or the possible mechanisms mediating these interactions, thus making it difficult to compare their study with the results of this thesis. There are still relatively a few studies including carnivores when examining interactions between spatially separated

organisms, and thus general conclusions or generalisations cannot be presumed.

The results obtained in Chapter 4 provide evidence of associational resistance between spatially separated herbivores, where *P. brassicae* 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). Futher 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, I considered interactions between root herbivores and parasitoids of foliar herbivores mediated by a plant that was shared by the consumers (root and foliar herbivores). Since these interactions were remarkably strong and root-feeding insects have shown to affect the growth and development of the parasitoid's off-spring, as well as the behaviour of female parasitoids foraging for hosts, I investigated whether they could also interact via the (micro) habitat they share. In **Chapter 5** I provide evidence that parasitoids of foliar herbivores find their hosts faster when the nearby plants are exposed to root herbivores than when the nearby plants are root undamaged. Therefore, root herbivores can indeed interact with aboveground parasitoids via the sahred habitat influencing the parasitoid's searching arena. These results suggest that spatially separated organisms can interact in much more complex and indirect ways than has been considered so far.

Previous studies have shown that structural changes in a habitat such as

plant height and plant composition (Gols et al. 2005), or herbivore species (McCann et al. 1998, Shiojiri et al. 2001, 2002, Vos et al. 2001) can influence parasitoid foraging efficiency. We used root-damaged and root-undamaged plants of similar shape and height to minimise the influence of physical plant characteristics on the foraging wasps. The results presented in this Chapter provide evidence that female parasitoids can exploit qualitative characteristics of the surrounding environment, triggered by belowground insects, to maximise their searching efficiency.

Aboveground-belowground root and foliar insect interactions in the field

In **Chapter 6** I show that root-feeding insects influence behavioural decisions of foliar-feeding insects that were attracted to *B. nigra* plants in the field. Overall, the specialised foliar-feeding insects preferred root-uninfested *B. nigra* plants as a food source and as an oviposition substrate, as opposed to plants shared with root herbivores. This case study provides evidence that in nature, where several complex interactions take place simultaneously, behavioural decisions of foliar-feeding insects can still be influenced by belowground insects. The distribution of the plants with root-feeding insects strongly influenced the behaviour of one of the aboveground species, *Brevicoryne brassicae*. This specialist aphid species exclusively preferred to attack root undamaged plants when the plants were grouped in clusters. Considering the influence of spatial distribution of the spatially separated organisms may be of crucial importance for some foliar-feeding insect species, and can influence the outcome of the experiments.

I previously observed (Chapter 4) that plants exposed to the root herbivore *D. radicum* provide a potential refuge for the foliar herbivore *P. brassicae*, since its parasitoid *C. glomerata* prefers to forage for *P. brassicae* hosts feeding on plants without root herbivores belowground. Unfortunately, naturally occurring *P. brassicae* butterflies were not observed ovipositing in the experimental field. Therefore, I cannot confirm that associational resistance between these

herbivores indeed takes place. It is important to bear in mind that in Chapter 2 I found that the performance of *P. brassicae* was suboptimal when sharing the host plant with root herbivores. *P. brassicae* is a gregarious insect that lays a few clutches of eggs per selected plant (Lemasurier 1994). Laying eggs on a suboptimal host plant (exposed to antagonistic root herbivores) will therefore imply a big penalty since several oviposition-events, with fitness costs, will usually occur on a plant. Therefore, mechanisms that increase the capacity of the foraging females to discriminate against plants shared with antagonistic root herbivores might also be expected to be favoured by natural selection. The preference of all the specialised foliar herbivore species for plants without root herbivores provide evidence that there is not associational resistance between the root herbivore *D. radicum* and the aboveground counterparts recorded on *B. nigra* plants in the experimental field.

The observation that aboveground herbivores avoid plants that experience root herbivory implies the interesting possibility that the avoidance behaviour of *C. glomerata* 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 and Van Dam 2005). Here I show, together with other contemporary studies (e.g. Omancini et al. 2001, Bezemer et al. 2005, Guerrieri et al. 2004), 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 aboveground 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 the understanding of terrestrial ecosystems requires an aboveground-belowground approach because of the strong feedbacks 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 and 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 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 observed that, in *B. nigra*, the volatile blend of root-damaged plants is characterised by high levels of sulphides and significantly differs from the volatile blend of root-undamaged plants. If there are also chemical or physical changes in the parasitoid-host on root-damaged plants that make the host less suitable, parasitoid females could potentially learn to associate the reduced quality of the 'reward' (the host) with the high concentration of

sulphides in the volatile blend of root damaged plants. Parasitoid females could then recognise from distance the presence of the root herbivores sharing the plant with its aboveground host. To date, it is unknown if root-feeding insects can influence learning in insect parasitoids. I think that this is a novel area of study. In Chapter 5, I showed 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 efficiently control pest-populations in agricultural systems. So far, research on refining and improving modern Integrated Pest Management programes has focused on experiments in which aboveground and belowground systems have been studied independently. The results of this thesis cannot be directly applied to improve biological control strategies, but suggest to look into the soil 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 foliar-feeding insects is extremely important to improve push-pull strategies in Integrated Pest Management programes in cropping systems. To date, Integrated Pest Management programes have been based on research focused on independent aboveground and belowground systems ignoring the fact that soil dwelling and aboveground insects may strongly interact.

The research carried out in this thesis aimed at evaluating and disentangling the feed-backs that occur between belowground 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 not very prolific 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, Wurst et al. 2006). Obviously, soil organisms interact with each other in many and complex ways, and this will affect the outcome of the effects in the host plant and consequently the impact on the aboveground food webs.

Finally, a major challenge in the field of aboveground belowground interactions is to generate models that capture these complex interactions and to validate their predictions under natural field conditions. In the meantime, we have to obtain reliable results from lab-based and field studies with naturally co-occurring species assemblages, with contrasting life histories, level of specialism and feeding guilds, without over-simplifying the systems because this will inevitably result in misleading conclusions.

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Summary

Herbivory can induce changes in plant growth, physiology, morphology, and phenology. Such herbivore-induced changes by insects are commonly observed in many plant species. Changes in the host plant can alter interaction linkages between plants, herbivores and higher trophic levels such as carnivores of the herbivores. These interaction linkages shape community organization and biodiversity in plant-based terrestrial communities. Considering that plants have both aboveground and belowground parts, systemic herbivore-induced changes within the plant could potentially link spatially separated herbivores associated with plants in the soil and in the aboveground (sub) system. Relatively recently, an increasing number of studies have shown that root herbivores, among other soil dwelling organisms, can indeed interact with foliar herbivores. Root herbivores change the quality and biomass of aboveground plant parts, which in turn influences the growth, development and survival of foliar herbivores. Similarly, foliar-feeding insects have been shown to influence root-feeding insects. This thesis explores whether, and mechanistically how, these feed-backs between root and foliar herbivores are transmitted through the soil and the aboveground food chains influencing the performance and behaviour of higher trophic levels.

In Chapter 1, I introduce plant-insect interactions in a multitrophic context, briefly describing how the plant mediates these intricate interactions, and I describe the general aim and the outline of the thesis. In Chapter 2 I study the effects of root herbivores, at different densities, on the biomass and quality of the plant shoot, and on the performance of a foliar herbivore, its primary parasitoid and its secondary parasitoid (hyperparasitoid) sharing the host plant. In Chapter 3 I study the reverse effect; the effects of foliar herbivores on a root herbivore and its parasitoid mediated by the host plant. The results of these Chapters show that the spatially separated herbivores negatively affect each other's performances by increasing the levels of secondary plant compounds within the host plant. These

negative effects are not restricted to the herbivore level, but are transmitted to the parasitoids and the hyperparasitoids, reducing in particular the growth and size of the off-spring of the aboveground parasitoid.

Based on the results of Chapter 2, where I observed a marked suboptimal performance of the off-spring of the aboveground parasitoid when sharing the host plant with root herbivores, I investigate in Chapter 4 whether female parasitoids of foliar herbivores can exploit root herbivore-related cues to select the most suitable hosts for their offspring. In this Chapter I show that female parasitoids avoid to parasitise hosts feeding on plants shared with root herbivores, when hosts feeding on root-undamaged plants are available. The mechanism points to changes in the volatile blend of the plant triggered by the root-feeding insects. After observing this strong interaction between root herbivores and aboveground parasitoids mediated by the host-infested plant, in Chapter 5, I investigate whether root herbivores influence the behaviour of female parasitoids also indirectly via changes in the surrounding habitat. I provide evidence that root-feeding insects can influence the behaviour and foraging efficiency of parasitoids of foliar herbivores, even when the foliar (parasitoid-host) and the root herbivores do not feed on the same plant, but on neighbouring conspecific plants within the same habitat.

The above described results are based on experiments carried out under controlled conditions in the laboratory or semi-field experiments in large, enclosed cages. To test some of the lab-based results under more natural conditions, I conducted a field experiment in which several cohorts of mustard plants with and without root herbivores were placed in the field and were monitored aboveground for the presence of foliar herbivores over the course of a summer season. To investigate whether the spatial distribution of root-damaged plants influences the plant preference of the foliar-feeders, I arranged the plants in the field in plots either in clusters of root-undamaged and root-damaged plants, or root-undamaged and root-damaged plants homogeneously mixed. Specialised foliar herbivores were observed feeding and ovipositing preferentially on root-

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uninfested plants over plants shared with root herbivores. Interestingly, one of the species only discriminates against root-damaged plants when the plants were grouped in clusters.

This thesis shows that the strong feedbacks between root and shoot associated organisms can significantly influence the performance and behaviour of organisms of higher trophic levels. Therefore, interactive effects of aboveground and belowground organisms cannot be limited to organisms directly associated with the shared host plant because they occur throughout a complex multitrophic chain of organisms. Furthermore, this thesis underlines the importance of considering not only the mere presence or absence of the soil dwelling organisms, but show that the density and distribution of root herbivores can determine the outcome of the interactions with their aboveground counterparts. These interactions are mediated by the shared host plant, via induced changes in the secondary plant compounds within the plant and in the plant volatile blend. Consumers in one compartment can change the quality of the plant in the opposite compartment, as well as induce changes in the emission of the plant volatiles used by carnivores that forage for hosts. Moreover, I provide evidence that spatially separated insects can interact not only via changes in the shared host plant, but also more indirectly via changes in the quality of the surrounding habitat. This suggest that aboveground-belowground multitrophic interactions can occur and interact in much more complex ways than what has been reported so far. In the last experimental Chapter I provide evidence that behavioural decisions in naturally occurring populations of foliar-feeding insects can also be influenced by root-feeding insects, under field conditions where several complex interactions take place simultaneously. In the final Chapter, I summarise the results presented in this thesis and provide a general conclusion of the work. I identify a number of important areas and topics for future research to further gain insight into aboveground-belowground multitrophic interactions.

Resumen

El daño causado por insectos puede inducir cambios en el crecimiento de las plantas, su fisiología, morfología y fenología. Estos cambios inducidos por herbívoros son comúnmente observados en diversas especies de plantas. A su vez, estos cambios en la plata hospedera pueden alterar las interacciones entre la planta, los herbívoros y demás niveles tróficos, incluyendo a los carnívoros de los herbívoros. Estas interacciones contribuyen a la organización y diversidad de las comunidades terrestres de plantas e insectos. La inducción sistémica originada por herbívoros en la planta puede potencialmente conectar herbívoros que se encuentran a distintos niveles en la planta, esto es, tanto en las raíces como en la parte aérea. Recientemente ha habido un incremento en los estudios que demuestran que organismos del suelo, incluyendo herbívoros, pueden interactuar con herbívoros presentes en las hojas de la planta. Herbívoros que se alimentan de las raíces de la planta pueden cambiar la calidad y biomasa del follaje de la planta y estos cambios pueden influenciar el desarrollo, crecimiento y mortalidad de los herbívoros del follaje. En una forma similar, herbívoros del follaje pueden afectar la performance de los herbívoros en el suelo. Esta tesis explora y analiza mecanísticamente cómo se dan estas interacciones entre herbívoros del suelo y herbivoros del follaje y su transmisión a lo largo de la cadena trófica, influenciando también la performance y el comportamiento de los niveles tróficos superiores incluyendo parasitoides e hiperparasitoides.

En el capítulo 1, se introduce el concepto de interacción planta-insecto en un contexto multitrófico, en el cual se ilustra brevemente estas complejas interacciones y se describe el objetivo general y el marco de la tesis. En el capitulo 2 se presentan los resultados de la evaluación de los efectos de los herbívoros del suelo que se alimentan de las raíces de las plantas sobre la biomasa y calidad del follaje de la planta y cómo esto afecta la performance de los herbívoros presentes en el follaje, sus parasitoides y sus hiperparasitoides. En el capitulo 3 se presenta la investigación realizada para evaluar el efecto reverso,

es decir el efecto de los herbívoros presentes en el follaje sobre la performance de los herbívoros localizados en las raíces así como sobre la performance de sus parasitoides. Los resultados de estos dos capítulos evidencian que herbívoros separados espacialmente, tanto en el suelo como en la parte aérea, afectan negativamente sus performances respectivas cuando comparten una misma planta hospedera. Este efecto está debido a un aumento en los niveles de compuestos secundarios en los tejidos vegetales de la planta. Otra conclusión importante es que este efecto negativo no esta restringido a los herbívoros que comparten directamente la planta, sino que se trasmite a través de la cadena trófica, afectando negativamente la performance de los parasitoides e hiperparasitoides comprometiendo en particular el tamaño y desarrollo de la descendencia de los parasitoides de los herbívoros del follaje.

Los resultados del capitulo 2, muestran un efecto de detrimento en la performance en la descendencia de los parasitoides de los herbívoros del follaje al compartir la planta hospedera con herbívoros de las raíces. En el capítulo 4 se investiga si los parasitoides pueden explotar señales relacionadas e inducidas por los herbívoros de las raíces para seleccionar los huéspedes más propicios para el desarrollo y crecimiento de su descendencia. Este capítulo muestra cómo las hembras adultas de los parasitoides evitan oviponer en las larvas que se alimentan del follaje de plantas compartidas con herbívoros de las raíces. El mecanismo que media estas interacciones parece estar relacionado con cambios en la composición y cantidad de compustos volátiles de la planta, cuya producción es inducida por los insectos que se alimentan de las raíces. Luego de observar esta fuerte interacción entre los insectos de suelo y los parasitoides de insectos del follaje, en el capítulo 5 se investiga si los insectos de suelo también pueden influenciar el comportamiento de los parasitoides que viven en la parte aérea a través de cambios, esta vez no en la planta hospedera, sino en plantas vecinas que hacen al ambiente que comparten. En este capítulo se presenta sólida evidencia de que los insectos que se alimentan de las raíces de las plantas pueden influenciar el comportamiento de los parasitoides de herbívoros del follaje

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incluso cuando el huésped del parasitoide, es decir el herbívoro del follaje, no comparten la misma planta sino que se alimentan de plantas vecinas dentro del mismo hábitat.

Los resultados descriptos anteriormente están basados en experimentos realizados bajo condiciones controladas de invernáculo y laboratorio o en estudios de campo dentro de tiendas. Para validar algunos de estos resultados provenientes de estudios en condiciones controladas en condiciones más naturales, se diseño un experimento a campo. En este experimento se distribuyeron en el campo experimental plantas de *B. nigra* con y sin herbívoros en las macetas, que fueron monitoreadas durante el verano para investigar la preferencia de herbívoros de follaje. Para investigar el efecto de la distribución espacial de las plantas con y sin herbívoros de raíces, las plantas fueron distribuídas alternadamente unas con otras o en grupos de plantas con y sin herbívoros en las raíces. Se observó que las especies de herbívoros de follaje especialistas prefirieron alimentarse y oviponer mayoritariamente en plantas no compartidas con herbívoros en las raíces. Una de estas especies discriminó en contra de plantas compartidas con insectos de raíces sólo cuando las plantas fueron distribuidas en grupos.

Los resultados de esta tesis muestran claramente que las interacciones entre insectos herbívoros de raíces y follaje pueden afectar significativamente la performance y comportamiento de insectos en niveles tróficos más altos que el inmediato y directamente relacionado con la planta. Por lo tanto, la interacción entre organismos asociados a raíces y follaje de una planta no necesariamente están limitados a aquellos organismos que directamente se alimentan o relacionan con la planta. Por el contrario, debido a que estas complejas interacciones ocurren y se trasmiten a lo largo de las cadenas tróficas del suelo y la parte aérea, su efecto pueden trasladarse a organismos más distantes en la cadena trófica. Además, los resultados de esta tesis enfatizan la importancia de considerar no solamente la presencia o ausencia de organismos del suelo, sino su abundancia y distribución como un factor clave que puede determinar los efectos en sus

contrapartes en la parte aérea. Estas interacciones entre insectos de suelo y raíces sobre varios niveles tróficos, son mediadas por cambios inducidos en los niveles de compuestos secundarios y volátiles de la planta. Los consumidores en un compartimiento, suelo o parte aérea, pueden inducir cambios en la calidad de la planta en el compartimiento opuesto, al mismo tiempo que pueden inducir cambios en los volátiles que los parasitoides utilizan para localizar a sus huéspedes. Insectos espacialmente separados pueden interactuar no sólo a través de la planta hospedera cuando la comparten, sino también a través de plantas vecinas presentes en un mismo hábitat. Esto evidencia que las interacciones multitróficas entre insectos de suelo y follaje pueden ser mas complejas de lo propuesto hasta el momento. En el último capítulo experimental se provee sólida evidencia que el comportamiento de poblaciones de herbívoros de follaje es influenciado por insectos que se desarrollan en el suelo y se alimentan de las raíces de las plantas, en la naturaleza, donde una innumerable cantidad de complejas interacciones ocurren simultáneamente. En el último capítulo se resumen los resultados presentados en los capítulos experimentales de la tesis y se provee una conclusión general del trabajo desarrollado en estos 4 años de investigación doctoral. Se identifican un número de áreas y temas donde se podría desarrollar futura investigación en esta área de trabajo para seguir avanzando en el conocimiento de las interacciones multitróficas entre insectos espacialmente separados y los complejos mecanismos que median estas relaciones.

Samenvatting

Vraat door plantenetende insecten kan veranderingen teweeg brengen in de groei, fysiologie, morphologie and fenologie van planten. Zulke veranderingen zijn regelmatig waargenomen, komen voor in een groot aantal plantensoorten, en kunnen invloed hebben op de interacties tussen planteneters en organismen hoger in de voedselketen zoals carnivoren. Dit kan weer invloed hebben op de organisatie en diversiteit van levensgemeenschappen, waarvan planten als primaire producenten de basis zijn.

Planten hebben bovengrondse en ondergrondse delen en vraat-gerelateerde veranderingen in de plant kunnen zowel boven als onder de grond plaatsvinden. Via zulke veranderingen in de plant kunnen bovengrondse en ondergrondse planteneters elkaar indirect beïnvloeden. Recentelijk zijn onderzoeksresultaten gepubliceerd die laten zien dat worteletende herbivoren en andere organismen die in de bodem leven, inderdaad invloed kunnen hebben op bladetende herbivoren. Wortelvraat veroorzaakt vaak veranderingen in de kwaliteit en kwantiteit van bovengrondse plantendelen en dit beïnvloedt de groei, ontwikkeling en overleving van bladeters. Er is ook bewijs dat bladeters, via hetzelfde principe, worteleters kunnen beïnvloeden. Dit proefschrift onderzoekt hoe zulke terugkoppelingen tussen ondergrondse en bovengrondse herbivoren plaatsvinden en hoe dit de groei en het gedrag beïnvloedt van carnivore organismen hoger in de voedselketen.

In Hoofdstuk 1 introduceer ik het begrip insect-plant interacties in een multi-troof perspectief. Ik beschrijf kort hoe de plant zulke interacties kan beïnvloeden en ik presenteer de algemene onderzoeksvraag en de opbouw van dit proefschrift. In Hoofdstuk 2 presenteer ik de resultaten van mijn onderzoek naar de effecten van worteletende insecten, in verschillende dichtheden, op de kwaliteit en kwantiteit van bovengrondse delen van de plant en op de ontwikkeling van een bladetende herbivoor, een sluipwesp (de natuurlijke vijand van de herbivoor) en een hypersluipwesp (de naturlijke vijand van de sluipwesp). In Hoofdstuk 3 bestudeer ik het omgekeerde: het effect van bladetende herbivoren, via veranderingen in de waardplant, op een worteletende herbivoor en een sluipwesp van deze worteletende herbivoor. De resultaten van deze hoofdstukken laten zien dat planteneters die in ruimte gescheiden zijn, elkaar negatief kunnen beïnvloeden via het effect van vraat op secundaire plantestoffen in de waardplant. Deze negatieve effecten zijn niet beperkt tot het eerste

trofische niveau, dat van de plantenteters, maar werken ook door op sluipwespen en hypersluipwespen. In het bijzonder werd de groei en grootte van nakomelingen van sluipwespen van bovengrondse planteneters negatief beïnvloed.

In Hoofdstuk 2 laat ik zien dat de nakomelingen van bovengrondse sluipwespen niet optimaal ontwikkelen als de waardplant blootgesteld is aan wortelvraat. Daarom onderzoek ik in Hoofdstuk 4 of volwassen sluipwespvrouwtjes kenmerken kunnen gebruiken die gerelateerd zijn aan wortelvraat om de beste gastheren te selecteren voor hun nakomelingen. In dit hoofdstuk laat ik zien dat sluipwespvrouwtjes vermijden gastheren te parasiteren die op waardplanten leven welke eveneens blootgesteld zijn aan wortelherbivoren, als er ook gastheren aanwezig zijn op planten met onbeschadigde wortels. De mechanistische verklaring lijkt te liggen in veranderingen in de samenstelling van vluchtige stoffen die de plant uitscheidt als gevolg van wortelvraat. Vervolgens onderzoek ik in Hoofdstuk 5 of worteleters ook het gedrag van sluipwespvrouwtjes kunnen beïnvloeden via veranderingen in de leefomgeving. Ik laat zien dat worteletende insecten het gedrag en de zoekefficiëntie van volwassen sluipwespvrouwtjes ook beïnvloeden als de bladeters en worteleters niet op dezelfde plant leven maar op buurplanten in dezelfde levenomgeving.

De tot-nu-toe beschreven resultaten zijn gebaseerd op experimenten die uitgevoerd zijn onder gecontroleerde omstandigheden in het laboratorium of in grote kooien. Om de betrouwbaarheid van de resultaten te testen onder meer natuurlijke omstandigheden, heb ik een veldexperiment uitgevoerd met verschillende groepen planten met en zonder wortelherbivoren. Gedurende een zomerseizoen heb ik de aanwezigheid van bovengrondse herbivoren gekwantificeerd. Om te bepalen of de waardplant voorkeur van de bladeters ook wordt beïnvloed door de ruimtelijke verdeling van planten met wortelschade, heb ik de planten in het veld geplaatst in groepen van planten met en zonder wortelschade en heb ik planten van deze twee groepen om en om geplaatst. Specialistische bladetende herbivoren waren vaker te vinden en legden meer eieren op planten zonder wortelherbivoren. Eén soort herbivoor vermijdde echter alleen wortelbeschadigde planten als planten met en zonder wortelschade in groepen waren geplaatst.

In mijn proefschrift laat ik zien dat de sterke terugkoppeling tussen wortel- en bladgeassocieerde organismen ook de ontwikkeling en het gedrag kan beïnvloeden van organismen die hoger in de voedselketen voorkomen. De interacties tussen

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bovengrondse en ondergrondse organismen kunnen daarom niet worden beperkt tot de organismen die direct gerelateerd zijn aan de waardplant, maar effecten kunnen ook zichtbaar zijn op andere plaatsen in de voedselketen. Mijn proefschrift onderstreept ook dat niet alleen de aanwezigheid of afwezigheid van organismen in de bodem bepalend is voor de interacties met bovengrondse organismen, maar ook hun aantal en ruimtelijke verdeling. Deze interacties vinden plaats via de gezamelijke waardplant, door veranderingen in secundaire plantenstoffen en door veranderingen in de samenstelling van vluchtige plantenstoffen.

Consumenten in één compartiment kunnen de kwaliteit van plantenonderdelen in het andere compartiment veranderen en veranderingen teweeg brengen in de uitscheiding door de plant van vluchtige stoffen die gebruikt worden door carnivoren voor het vinden en selecteren van gastheren. Bovendien laat ik zien dat insecten die in ruimte van elkaar gescheiden zijn, elkaar niet alleen beïnvloeden via de gezamelijke waardplant, maar ook, op een indirecte manier, via veranderingen in de kwaliteit van de leefomgeving. Deze resultaten suggereren dat bovengrondse-ondergrondse multi-trofe interacties veel vaker voorkomen dan wat tot nu toe is gedocumenteerd.

In het laatste experimentele hoofdstuk laat ik zien dat ook in natuurlijke omstandigheden, worteletende insecten het gedrag van bladetende insecten beïnvloeden. In het laatste hoofdstuk vat ik de resultaten van dit proefschrift samen en kom ik tot een algemene conclusie. Bovendien identificeer ik een aantal belangrijke onderzoeksvragen voor vervolgonderzoek om meer inzicht te verwerven in bovengrondse-ondergrondse multi-trofe interacties.

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want to specially thank you for your support in the last phase of putting together all our research and building up this thesis-book, where you read each piece of text as many times as I asked you. Wim, thank you for being an extraordinary close 'promotor', with your door always open at any time that I was passing by saying 'un minuto' for a question. You always had time to talk and even read proposals for future research. You were always very open to suggest and leave the final decisions to me, thanks! Louise, I want to thank you for being always available for me, in person when you were around and by e-mail and telephone when you were not at the NIOO or abroad. In Spanish I would say that you have 'la sencillez de los grandes', which more or less means that you have 'the simplicity of those that are truly brilliant'. I admire you as a researcher and as a woman. You always promote women in science deeply from your heart, and that is, I am certain, very much appreciated by women like me that are starting in science.

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All my interest for insects is the result of a long process that started more than a decade ago in Uruguay when I joined the Entomology Department of my University, University of Agronomy, when I was still a student. There I developed my interest for insects, my interest to understand them at that time with the direct aim of tackling pest problems in fruit trees. Thanks Beatriz for giving me the chance to work with you and for all the support and encouragement to develop my career in entomology together with you and Nino at the National Institute of Agricultural Research of Uruguay. Bea, your support

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Curriculum vitae

Roxina Soler was born on 16th November 1972 in Montevideo, Uruguay. In 1999, she obtained the degree of Ingeniera Agrónoma (Agronomist Engineer) at the Universidad de la República, Montevideo, Uruguay. During her studies in 1995, she joined the Department of Entomology of the same University. Her activities included both research and undergraduate teaching in Entomology and Plant protection. Research focused on the biology and seasonal development of insect pests in fruit trees in Uruguay, with the objective of developing environmental-friendly control strategies in commercial production. This research involved close interactions with farmers. In 1996 and 1997 she participated in a project to evaluate alternative control measures using attractant-chemicals of a native pest of apple trees in Uruguay. The project was run in collaboration with the Department of Entomology and Pharmacognosy. In 1998, she also joined the Plant Protection Department of INIA (National Institute of Agricultural Research). Here she investigated the biology and seasonal development of insect-pests in vineyards, and in the development of control strategies in commercial production.

In 2001, she started an MSc program in Plant Science at Wageningen University, which was completed in 2003. In her MSc thesis, which was done at the Laboratory of Entomology she studied the reproduction and searching behaviour of a natural enemy of whiteflies in the frame of a biological control program. The thesis work was supervised by Prof. Joop Van Lenteren. In 2003, she conducted a three-month study with Dr. Nina Fatourous at the same department, studying the response of a generalist egg parasitoid to plant volatiles induced through oviposition by one of its hosts. During her work at the Laboratory of Entomology, she became interested in the behavioral ecology of insect parasitoids and in understanding multitrophic interactions. This interest was continued and intensified during her PhD research, which was conducted at the Netherlands Institute of Ecology (NIOO), Centre of Terrestrial Ecology, between October 2003 and October 2007. The research focused on the study of multitrophic interactions between soil dwelling and aboveground insect herbivores and primary and secondary parasitoids, and was supervised by Drs. Jeff Harvey, Martijn Bezemer, and Profs. Wim Van der Putten and Louise Vet.

List of Publications

Peer-reviewed

Soler R, JA Harvey and TM Bezemer. 2007. Foraging efficinecy of a parasitoid of a leaf herbivore is influenced by root herbivory on neighbouring plants. *Functional Ecology* 21: 969-974.

Soler R, TM Bezemer, AM Cortesero, WH van der Putten, LEM Vet and JA Harvey. 2007. Impact of foliar herbivory on the development of a root herbivore insect and its parasitoid via changes in host plant quality. *Oecologia* 152: 257-264

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JA Harvey, NM van Dam, LMA Witjes, **R Soler** and R Gols. 2007. Effects of dietary nicotine on the development of a herbivore, its parasitoid and secondary hyperparasitoid over four trophic levels. *Ecological Entomology* 32: 15-23.

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NE Fatouros, G Bukovinszki'Kiss, LA Kalkers, **R Soler**, M Dicke and M Hilker. 2005. Oviposition-induced plant cues: do they arrest *Trichogramma* wasps during host location? *Entomologia Experimentalis et Applicata* 115: 207-215.

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Aleyrodidae). *Proceedings of the Netherlands Entomological Society meeting* 15: 111-117.

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Soler R, S Nuñez and I Scattoni. 1999. Mealybugs behavior during vineyard dormancy. Advances in research on Mealybugs. Extension Meeting. *National Agricultural Research Institute of Uruguay (INIA).* Publisher: INIA Montevideo.

Soler R, I Scattoni and S Nuñez. 1999. Control of "Red scale" in Apple trees. Experimental results in crop protection in fruit trees and vineyards. Technical bulletin. *National Agricultural Research Institute of Uruguay (INIA).* Publisher: INIA Montevideo.

Scattoni I, G Asplanato, C Bentancour, J Pazos, **R Soler**, J Franco and J Paullier. 1999. Population dynamics of the Citrus Leaf Miner *Phylliocnistis citrella* in Uruguay. Technical bulletin. *National Agricultural Research Institute of Uruguay (INIA).* Publisher: INIA Salto.

Scattoni I, S Nuñez and **R Soler**. 1999. Seasonal development of mealybugs in vineyards. Second year of evaluation. Technical Bulletin. *National Agricultural Research Institute of Uruguay (INIA).* Publisher: INIA Montevideo.

Prizes and Awards

Elton Prize. Prize awarded for the best paper by a young author published in *Journal of Animal Ecology* in 2005 to: **Soler R**, TM Bezemer, WH van der Putten, LEM Vet and JA Harvey. 2005. Root herbivore effects on aboveground herbivore, parasitoid and hyperparasitoid performances via changes in plant quality. *Journal of Animal Ecology* 74: 1121-1130.

Storm-van der Chijs Award. Prize that distinguishes young female scientists for their international and interdisciplinary approach in their research.

PE&RC PhD Education Certificate

With the educational activities listed below the PhD candidate
has complied with the educational requirements set by the C.T. de Wit
Graduate School for Production Ecology and Resource Conservation
(PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of
activities)

Review of Literature (5.6 credits)

- Linking above-belowground multitropic interactions (2003)

Writing of Project Proposal (5.6 credits)
- Linking interactions between above- and below- ground herbivores and the performance of parasitoids and hyper-parasitoids (2004)

Laboratory Training and Working Visits (1.4 credits)
- Learning mass-rearing of *D. radicum*; Rennes University (France)

Post–Graduate Courses (5.2 credits)

- Scientific writing in English; PE&RC (2005)
- Behavioural ecology of insects parasitoids; BEPAR (2005)
- Soil ecology: crossing the frontier between below and aboveground; PE&RC (2007)

Discussion Groups / Local Seminars and Other Scientific Meetings (9.8 credits)

- Multitrophic interactions weekly discussion group (2003-2007)
- PhD meeting group; twice per month (2003-2007)

PE&RC Annual Meetings, Seminars and the PE&RC Weekend (0.9 credits)

- PE&RC weekend (2004)

International Symposia, Workshops and Conferences (10.3 credits)

- Netherlands Entomological Society Meeting (NEV); Groningen, the Netherlands (2004)
- Direct interactions in the rhizospere; Wageningen, the Netherlands (2005)
- International Botanical Congress; Vienna, Austria (2005)
- IX European Workshop on Insect Parasitoids; Cardiff, UK (2005)
- British Ecological Society Annual Conference; Oxford, UK (2006)
- Behavioural Ecology of Insect Parasitoids; France (2006)
- Plant-Insect Interactions; Knvista, Sweden (2007)
- X European Workshop on Insect Parasitoids; Sicily, Italy (2007)

Courses in Which the PhD Candidate Has Worked as a Teacher

- Communication and integration in biological systems; Plant Ecology, Nijmegen University; 1 day

Supervision of MSc Students

- Above-belowground insect interactions; 4 students supervised