



Nature, Evolution and Characterisation of Rhizospheric Chemical Exudates Affecting Root Herbivores

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Abstract

Similar to aboveground herbivores, root-feeding insects must locate and identify suitable resources. In the darkness of soil, they mainly rely on root chemical exudations and, therefore, have evolved specific behaviours. Because of their impact on crop yield, most of our knowledge in belowground chemical ecology is biased towards soil-dwelling insect pests. Yet, the increasing literature on volatile-mediated interactions in the ground underpins the great importance of chemical signalling in this ecosystem and its potential in pest control. Here, we explore the ecology and physiology of these chemically based interactions. An evolutionary approach reveals interesting patterns in the response of insects to particular classes of volatile or water-soluble organic compounds commonly emitted by roots. Food web analyses reasonably support that volatiles are used as long-range cues whereas water-soluble molecules serve in host acceptance/rejection by the insect; however, data are still scarce. As a case study, the chemical ecology of *Diabrotica virgifera virgifera* is discussed and applications of belowground signalling in pest management are examined. Soil chemical ecology is an expanding field of research and will certainly be a hub of our understanding of soil communities and subsequently of the management of belowground ecosystem services.



1. INTRODUCTION

Research on plant–insect interactions has historically focused on aboveground niches where numerous interactions among various functional guilds (e.g. herbivores, pollinators, predators and parasitoids) have been described. Decades of research exploring almost all terrestrial ecosystems, ranging from complex natural meadows or native forests to monoculture cropping systems, have shown that arthropods rely on plant-derived cues that are either physical (visual or tactile) or chemical (olfactory or gustatory) to identify, locate and finally accept or reject plants (e.g. [Schoonhoven et al., 2005](#) and references therein). From the perspective of mankind, such interactions can be positive (i.e. pollination by insects) or negative (i.e. yield reductions) and attempts to respectively favour or control certain plant–insect interactions have been conducted in order to optimise (agro-) ecosystem functions and services.

In comparison, over a similar period of time, belowground plant–insect interactions have been largely understudied (Hunter, 2001). Indeed, an informal assessment by Hunter (2001) suggested that insect–root herbivory represents 2–5% of the literature published on insect herbivory, whereas plants from most ecosystems allocate 50–90% of their net primary production in root systems (Coleman, 1976). That the collective biomass of root–xylem-feeding cicadas is greater than any other animal in terms of biomass per unit area in certain North American forests (Karban, 1980) or that the weight of sheep per hectare is exceeded by the biomass of chafers in some Australian pastures (Britton, 1978) also illustrate well the importance of belowground insect herbivory. As hypothesised by Hunter (2001), soil-dwelling insects and roots are difficult to access, which likely contributes to most scientists' limited attention despite the clear linkages of below- and aboveground processes.

However, during the last decades, root–insect and root–microorganism interactions have drawn increasing consideration (e.g. Bonkowski et al., 2009; De Deyn et al., 2003; De La Peña et al., 2006; Gange and Brown, 2002; Rasmann and Agrawal, 2008; Rasmann et al., 2012b; Strong et al., 1999; van Dam, 2009; Van Der Putten, 2003). Because of their evident impact on human societies, most of the rhizophagous research has been conducted on insect pests of crops (Johnson and Murray, 2008). However, not all root herbivory is detrimental to human interests. Indeed, a total of 49 root–herbivore insect species were released in nearly 100 attempts to control 19 different target plant pest species in 10 different countries (Blossey and Hunt-Joshi, 2003 and references therein). The weevil *Cyphocleonus achates* (Coleoptera: Curculionidae) reduces the biomass production and fitness of spotted knapweed *Centaurea stoebe* (Asterales: Asteraceae) (Knoche and Seastedt, 2010), an invasive weed in North America. The effectiveness of biological control by the weevil was, however, compromised by biotic (plant competition) and abiotic (soil resources) factors that ultimately favoured the targeted pest plant (Knoche and Seastedt, 2010). Yet, the application of root herbivory in weed control still requires optimisation (Ortega et al., 2012) and with respect to environmental safety, in-depth studies have to be conducted before a root herbivore can be mass-released to control invasive weeds such as knapweed. Nonetheless, Blossey and Hunt-Joshi (2003) concluded that root feeders are more likely to contribute to weed control than aboveground-dwelling insect herbivores.

Just as with aboveground plant–insect interactions, plants can also interact with rhizospheric insects without deleterious effects. Such commensal association has been described in the interaction between the broadleaf

cattail (*Typha latifolia*, Typhales: Typhaceae) and *Coquillettidia* species (Diptera: Culicidae) (S erandour et al., 2008). Indeed, the aquatic larvae of these insects rely on root aerenchymes as a source of oxygen in benthic anoxic substrate (Bosak and Crans, 2002) and therefore respond to water-soluble secondary metabolites released by its host plant to locate an adequate site of establishment (Bosak and Crans, 2002). This nondestructive relationship between roots and insects might provide the insect not only with oxygen but also with better sources of food. For instance, the extensive rhizospheric exudation of organic matter by the plant could favour the abundance and diversity of microorganisms on which the mosquito larvae feed (Merritt et al., 1992). This increase in nutrient turnover might also be beneficial to the plant (Bonkowski, 2004 and references therein), but this has not been tested in this particular context.

The theoretical and evolutionary concepts pertaining to how above-ground chemically mediated plant–insect interactions are structured (Karban and Baldwin, 1997; Price et al., 1980; Vet and Dicke, 1992) also apply to subterranean systems (e.g. Rasmann et al., 2011a; Strong et al., 1999; Van Der Putten et al., 2009; van Tol et al., 2001). Indeed, roots impact the behaviour, composition and abundance of belowground communities (e.g. Bonkowski et al., 2009) and their trophic links (Coleman, 1976; Monroy and van der Putten, 2009; Strong et al., 1999). However, conspicuous differences (physical and mechanistic) appear between these two broad ecological niches.

1.1. Soil versus air

Differences between soil and air characteristics are, to some extent, easy to conceive but imply dramatic divergences in their respective physical and chemical properties. At a glance, air is mainly composed of one phase of matter (gas), whereas soil is constituted of all three (gas, liquid and solid) (Fig. 3.1). Despite its apparent simplicity, atmospheric air composition plays an important role in chemically mediated interactions between insects and plants aboveground. Oxygen is highly chemically reactive and can therefore oxidise volatile organic compounds emitted by plants. Differences in temperature, water vapour levels and pressures result in diverse volatile organic compound diffusion characteristics on a micro- or macroscale.

However, the troposphere (75–80% of the atmosphere total mass over a height of 10–15 km) can be considered homogenous as compared to soils covering the surface of the globe. Indeed, adding two extra phases of matter

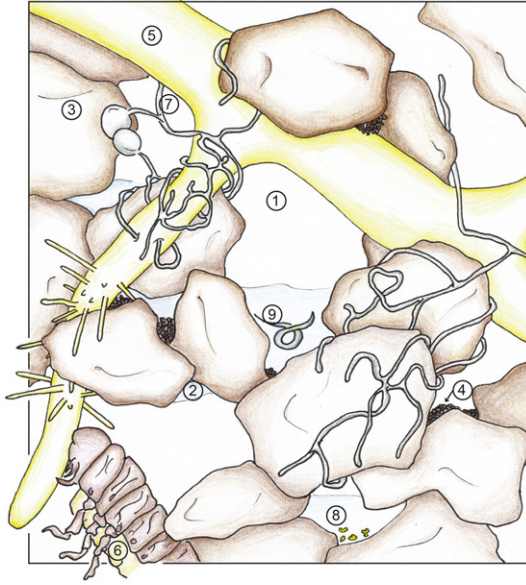


Figure 3.1 Despite an apparent homogeneity, soil is a very complex medium. Besides atmospheric air (20–30% of the total composition) filling up pores (1), water is present either in the gaseous phase or as a liquid ((2), 20–30% of the total composition), influencing both biotic and abiotic conditions. The mineral portion of soil ((3), 45% of the total composition) varies not only with the basal rock characteristics it originated from but also with external outcomes brought along by wind or water. Organic matter (4) represents a portion of about 8% of the total composition of soils and plays a major role in the formation of the clay–humic complexes that are unique to soils and ultimately allow life to proliferate. The soil biota is spanning from microorganisms to mammals. Plant roots (5) are an important component of soil biota, as they constitute a sink of carbon for insect herbivory (6) or other interaction with fungi (7) or microorganisms (8) such as nematodes (9). The diversity of the encountered organisms and the diversity of the holding media make interactions in soil very complex and extensively diverse. Chemical cues between soil-dwelling organisms are an important component of their communication arsenal. *Drawing: I. Hiltbold.*

offers considerably more variation and therefore an increased diversity in soil types and properties (see also [Chapter 1](#)). Soil diversity is characterised by several properties. (1) The soil texture is probably the soil's physical property that has the most profound impact on other soil properties and on soil biota. Defined as the proportion of the three mineral particles, *sand*, *silt* and *clay*, texture results from basal mineral breakdown and exogenous deposition of material by wind and/or water. (2) Soil structure is the arrangement of the soil particles in aggregates. The structure of a soil will impact its stability,

porosity and water regime and therefore determine the possible uses of a particular portion of land. Soil structure is classified in seven different types, such as granular or prismatic, that depend on how soil components clump together. (3) Soil porosity, defined as air-/water-filled spaces between particles and aggregates, is the portion of soil where several biotic processes take place. The two previously described properties influence the size, the number and the interconnection of pores. Soil porosity is not constant and can be modified by management procedures and/or land use and has an influence on the soil density. (4) Chemical activity in soils is extremely high. Indeed, in addition to chemical reactions occurring in the aerial or liquid phase of soil, several chemical processes and interactions take place on the surface of the colloids, the finest clay and organic matter particles (clay–humic complex). All these properties, along with several biotic and abiotic processes, result in a highly fragmented environment. Vertical soil diversity ranges from loose litter to the basal rock and is organised in “horizons”. As a result of the variability, soil horizons offer several different niches impacting species diversity, behaviour and potential interactions with roots. Horizontally, soil can be relatively homogeneous over a particular surface (i.e. agricultural land) or can dramatically vary depending on local parameters such as topography, water regime and vegetation. These variations affect both the diffusion of chemical signals (Hiltbold and Turlings, 2008) and the related behaviour of the insects (see Chapter 1). As pointed out by Johnson and Gregory (2006), a volatile diffuses more rapidly through 1 m of air than through 1 mm film of water covering soil pores (Payne and Gregory, 1988).

These various soil parameters impact behaviour, mobility and signalling, therefore altering interactions between soil-dwelling organisms in a dramatically different manner than air would in aboveground ecosystems. Indeed, soil organisms (excluding those living in litter) usually do not explore more than a 1 m² during their lifespan, whereas above-ground, some insects can wander through several times that surface within 1 day (Hedlund et al., 2004). Chemical properties of soil suggest higher metabolite retention capacity and, therefore, more stable chemical gradient establishment allowing slow-motion soil organisms to locate conspecifics or food resources. As compared to plant shoot physiology, roots evolved particular physiology, potentially leading to very distinct interactions with herbivores in soils (Erb et al., 2012).

Despite these differences between above- and belowground environments, these two ecospheres should not be seen as two separate and independent milieus. Indeed, besides the obvious physical link made by plants

crossing the “border” between both spheres, many heterophagous insects spend different parts of their life stages in both environments (e.g. notably Coleoptera and Diptera species) or inhabit both as adults (e.g. Isoptera species) (Brown and Gange, 1990). A growing literature describes above-ground–belowground interactions (e.g. Johnson et al., 2012, 2013; Rasmann and Turlings, 2007; Soler et al., 2007; van Dam et al., 2003, 2004, 2005). As compared to aboveground insects that rely on olfactory cues for long-distance orientation and visual stimuli at a closer range (e.g. Otálora-Luna et al., 2013), soil-dwelling insects possibly do not use visual cues at all and rather chemical or tactile/gustatory cues affect their behaviour and interactions with their surrounding environment (Johnson and Gregory, 2006; Johnson and Nielsen, 2012).

1.2. Root exudation of chemical compounds in the rhizosphere

Thousands of papers report on plant metabolites and their impact on insect ecology and evolution. Indeed, it is commonly accepted that plants produce over 200,000 different compounds (Dixon and Strack, 2003), historically known as “secondary metabolites” but more recently redefined as “specialised metabolites” (Pichersky et al., 2006; Schilmiller et al., 2008; Yonekura-Sakakibara and Saito, 2009). Since the pioneer work by Fraenkel (1959), a wide diversity of compounds and biosynthetic pathways have been described and elucidated (e.g. Pichersky et al., 2006). This striking number of known plant-produced compounds is likely an underestimate. Only a few plant species have been investigated for their production of specialised metabolites, and some compounds are present only in minute quantities that prevent their isolation or detection. However, new methods and specialised equipment, which facilitate in identifying new chemicals, further help to elucidate new biochemical pathways and the ecological relevance of plant metabolites (e.g. Glauser et al., 2013). Care must be taken with these techniques, however, as sample preparation and the instability of some compounds can erroneously produce molecules that are not originally present in plants. Currently, most studies evaluating how root exudates shape their environment have been on agricultural pests such as root-feeding insects (Johnson and Gregory, 2006; Johnson and Nielsen, 2012), plant parasitic nematodes (Rasmann et al., 2012a) or other microorganisms (e.g. Akiyama et al., 2005). Nevertheless, roots synthesise a similar diversity of specialised metabolites as compared to plant shoots (Kaplan et al., 2008). This metabolite diversity is reflected in traditional Chinese medicine where

more than one-quarter of the pharmacopoeia were derived from below-ground plant material (Bensky and Gamble, 1986).

As discussed in the previous section, soils simultaneously offer two phases for root exudates to diffuse through connected pores: a gaseous phase composed of air and a liquid phase composed of water and water-soluble molecules and ions. This provides the opportunity for soil-dwelling insects, much as other belowground organisms, to exploit volatile organic compounds or water-soluble organic compounds for long-distance detection, local orientation and food acceptance. The role of root-exuded metabolites on soil biota and biotic interactions has been discussed in seminal reviews (Badri et al., 2009; Bais et al., 2006; Bonkowski et al., 2009; Flores et al., 1999; Hiltbold and Turlings, 2012; Rasmann et al., 2012b; van Dam, 2009; Wenke et al., 2010), and growing interest in such interactions will likely lead to a rapid increase of our knowledge. Here, we focus on the interaction between insect and roots, their evolution and potential applications in an agricultural context.

1.3. Root-mediated chemical identification and selection by insects

Plants can release up to 20% of their fixed carbon via exudation through roots (Barber and Martin, 1976). This release of organic metabolites can affect abiotic and biotic soil conditions, and there is increasing evidence that insect behaviour is partially mediated by root exudates. In a comprehensive review, Johnson and Gregory (2006) proposed a seminal schematic model of the importance of plant metabolites in the location and selection of root by insects. Further discussed by Hiltbold and Turlings (2012) and Chapter 2, this model offers a detailed illustration of the different steps affecting insect behaviour during root foraging. First, foraging insects shift from a random to biased-random movement. Such a shift is most likely triggered by carbon dioxide (CO₂) (Johnson et al., 2006). This ubiquitous compound plays a similar role aboveground (Dekker et al., 2005; Turner et al., 2011) as it does with soil-dwelling organisms (Dillman et al., 2012b; Turlings et al., 2012). Certain insects can detect very low variations in CO₂ concentrations (Bernklau and Bjostad, 1998a; Doane et al., 1975; Klinger, 1958) enabling them to follow gradients. As a result, CO₂-biased movement will likely bring the insect into contact with more specific plant cues such as plant-emitted volatile organic compounds and/or water-soluble organic compounds (Johnson and Nielsen, 2012). Either the volatile organic compounds or the CO₂ gradient eventually leads the foraging insect to the plant tissue.

On a finer scale, insects may rely on water-soluble organic compounds to locate their plant host (Bernklau and Bjostad, 2008; Hibbard et al., 1994). Microbes interacting with roots also shape interactions in the rhizosphere (e.g. Cipollini et al., 2012) and therefore can affect insect behaviour (Vannette and Rasmann, 2012). Insects themselves carry chemical-producing symbiotic microbes (e.g. Dematheis et al., 2012) and can additionally facilitate such interactions.

Even though this does not well fit the scope of the present discussion, it is interesting to mention that vibrations are important physical cues for subterranean insects. This growing field of research increases our fundamental knowledge in insect ecology and potential applications in pest control (e.g. De Groot et al., 2011; Ennis et al., 2010; Eriksson et al., 2012; Kojima et al., 2012; Laumann et al., 2013; Mankin, 2012; Mukai et al., 2012; Tokuda et al., 2010), in plant physiology (Gagliano et al., 2012b) and in root ecology (Gagliano et al., 2012a). The conjugated effects of both acoustic and chemical signals on the behaviour of soil-dwelling insects require further research to document the respective and/or cumulative effect of both cues. Since plants can detect vibrations (Gagliano, 2013), those caused by insects in the rhizosphere might result in shifts of specialised metabolite synthesis and exudation to directly defend the plant tissues or to trigger indirect defence pathways.

1.4. Aims and scope

The present discussion aims to provide an overview of the insect physiology of chemical perception and environment-specific adaptation in subterranean insects. The evolution of belowground insect chemotaxis will be discussed. Whereas insects rely on chemical cues to locate their food source, they also emit volatiles that can be used as location cues by natural enemies (e.g. Dillman et al., 2012b) or they could induce shifts in root volatile organic compound profiles resulting in the attraction of such natural enemies (e.g. Hiltbold et al., 2011; Rasmann et al., 2005). Evolutionary aspects of these interactions are still scarce but could help in drawing general hypotheses and theories on soil community interactions, even though there is a strong bias towards agricultural insect pests in our actual knowledge. An update on newly released methodologies to study belowground insect behaviour and physiology in relation to roots is also proposed. Indeed, recent developments are offering root biologists new powerful approaches to disentangle root interactions with their surrounding belowground communities. Because of their economic and

social impact on human societies, most of the belowground research focuses on root pests mainly from the Coleoptera and Diptera orders. Therefore, a detailed example of root pest chemical ecology is finally discussed, using Western corn rootworm (WCR) (*Diabrotica virgifera virgifera* LeConte, Coleoptera: Chrysomelidae) as a model species, and we discuss the potential of chemical ecology in managing soil-dwelling insect pests.



2. OLFACTION IN SOIL-DWELLING INSECTS

As for any other organism, insects rely on a wide range of sensory systems not only to locate and evaluate food but also to shelter, mate or avoid threats such as predation and harmful abiotic environments. Smelling their surrounding direct and distal environment is of pivotal importance for insects (Dethier, 1947) even though it can be sometimes subordinate to vision in aboveground species such as *Diaprepes abbreviatus* (Coleoptera: Curculionidae) (Otálora-Luna et al., 2013). Yet, the necessity of olfaction reflects the wide range of antennal structures that both aboveground and, to some moderate extent, belowground insects possess. In addition to their antennae, insects also evolved other organs to detect chemical compounds such as maxillary or labial palps (Schneider, 1964). However, the necessity for multiple types and locations of olfactory organs still remains unclear. It can be hypothesised that both antennae and palps are designed to sense different sorts of compounds, as in the malaria vector *Anopheles gambiae* (Diptera: Culicidae), which has palpal sensillum extremely sensitive to CO₂ and 1-octen-3-ol (Lu et al., 2007), two volatile organic compounds usually emitted by its hosts. However, some other species exhibit overlapping in odour detection by antennae and palps. The vinegar fly *Drosophila melanogaster* (Diptera: Drosophilidae) accomplishes CO₂ detection with both organs as their respective olfactory sensory neuron activity overlaps (De Bruyne et al., 1999). The reason for this remains unclear but is very likely to result from selection for high sensitivity and phylogenetic and/or developmental limits and abiotic constraints (Hansson and Stensmyr, 2011). This ability to accurately smell the environment and the evolutionary plasticity of this trait may explain why insects are so abundant and diverse in heterogeneous ecological niches, like soils.

2.1. Molecular mechanisms of odour detection by insects

Sensory organs in insects come in multiple shapes and sizes and are located in several places on the insect body. Nonetheless, mechanisms of odour capture

and further identification are very similar. Molecular responses of insects to odorant cues are characterised by two large and exclusive gene families coding for (1) the odorant-binding proteins (Vogt and Riddiford, 1981) and (2) odorant receptors (Clyne et al., 1999; Vosshall et al., 1999). Odorant-binding proteins are produced and secreted in large quantities in the lymph surrounding the olfactory sensory neuron (Swarup et al., 2011). The exact role of these proteins still remains to be elucidated, but it is widely hypothesised that odorant-binding proteins are involved in the transport of odour ligands to the receptor site on the neural dendrites (Hansson and Stensmyr, 2011) when volatiles reach the sensillum lymph via pores or slits in the sensillum cuticle (Steinbrecht, 1997). Similar proteins have also been described in mammals (Vieira et al., 2007); however, they are structurally dissimilar. Hence, insect odorant-binding proteins could be as old as insects themselves and might have evolved to face the dry land conquest constraints (Vieira et al., 2007). The contact between the odour stimuli and the odorant receptors on the surface of the olfactory sensory neuron membrane triggers the production of signals in the dendrite, which are then transmitted to the insect's brain. Similar to the odorant-binding protein gene family, odorant receptor genes, coding for the neural receptors, are also exclusive to insects (as well as insect gustatory receptors) (Clyne et al., 2000; Scott et al., 2001). Although odorant receptors are more documented in comparison to odorant-binding proteins, the evolution and diversification of odorant receptor gene family remains uncertain (Hansson and Stensmyr, 2011 and references therein). In some species, such as the *D. melanogaster* (Kondoh et al., 2003), a sexual brain dimorphism evolved to respond to the necessity of males to detect pheromones to identify reproductive conspecifics.

Even if many evolutionary and physiological aspects still need to be clarified, insects have the ability to detect odours using very specific and fine-tuned morphological and physiological adaptations. Olfactory machinery has evolved to specifically respond to ecological constraints encountered by root-feeding insects.

2.2. Smelling the dark

Most of the studies on insect olfaction focus on the olfactory perception of their environment by aboveground insects. Therefore, even though it is likely to exhibit similarities, little is known about the sensory appendages and olfactory physiology of soil-dwelling insects or their larvae. Yet, because of their economic impact, studies on different rhizophagous species have

been conducted, mainly focusing on the detection by insects of the ubiquitous CO₂ signal (e.g. Cobb, 1999; Doane and Klingler, 1978; Giglio et al., 2003; Jepson, 1937; Keil, 1996; Klinger, 1957; Stange and Stowe, 1999; Sutherland and Hilier, 1974). A comprehensive study was recently published by Eilers et al. (2012) where they demonstrated that belowground insects are able to physiologically discriminate several volatile organic compounds. After an in-depth description of the sensory structure of the European cockchafer *Melolontha melolontha* (Coleoptera: Scarabaeidae) larva, the authors tested the olfactory responses of these appendices to compounds likely to be present in the rhizosphere such as host plant kairomones (Eilers et al., 2012). In a series of elegant experiments, they demonstrated that the chafer larva has a highly developed chemosensory apparatus and responds to a wide range of compounds typically emitted by plants and potentially by plant roots. Moreover, each olfactory structure showed a very specific response profile to various stimuli with some discriminations down to the level of molecular chirality (Eilers et al., 2012), suggesting a very well-developed sense of smell, even though appendices are more much discreet than similar ones on aboveground insect herbivores (Eilers et al., 2012). Narrowness of soil pores and cracks also limits the development of delicate olfactory appendices such as those sometime encountered aboveground. In a similar study on the congeneric forest cockchafer *M. hippocastani* (Coleoptera: Scarabaeidae), Weissteiner et al. (2012) reported an antennal lobe composed of around 70 glomeruli. This brain complexity reflects the diversity of the odorant receptor proteins and consequently the olfactory sensory neuron (Stocker, 1994) and is comparable to the well-established complexity described in aboveground adult model insects (e.g. Grosse-Wilde et al., 2011; Stocker, 2001). Because of its extended development time spent in soil, the cockchafer could have evolved a very complex chemosensory apparatus that may not be shared with all subterranean insect larvae. Yet, other species possess well-developed olfactory appendices (Cobb, 1999), and it is likely that the knowledge of the olfactory physiology of soil-dwelling insects will increase in the future.



3. EVOLUTION OF INSECT CHEMOTAXIS IN ROOT-INSECT INTERACTIONS

3.1. Adopting an evolutionary approach

Chemotaxis is generally defined as a directed behaviour towards or away from a source of chemical stimuli. In this section, we summarise knowledge

on major attractants and phagostimulants for foraging root–insect herbivores. Whereas CO₂ plays an important role in subterranean chemotaxis (e.g. Turlings et al., 2012, and Section 5 of the present discussion), it is a ubiquitous belowground cue that most organisms studied respond to (e.g. Johnson and Gregory, 2006; Johnson and Nielsen, 2012; Rasmann et al., 2012a and Section 5) and therefore was excluded from the following analyses.

Using phylogenetic tools and food web analytic approaches, evolutionary patterns emerged from the published literature on insect–root–positive chemotaxis or host acceptance, even though they were biased towards crop insect pests (Tables 3.1 and 3.2). Briefly, the phylogeny was based on gene sequences publicly available on GenBank for three nuclear DNA regions (18S rRNA (KC177301.1, KC177311.1, AF515703.1, AF308339.1, AY244865.1, AY988454.1), 28S rRNA (DQ198733.1, KC177741.1, KC177819.1, FJ867676.1, AF308387.1, JX263722.1, AY243741.2, GU226589.1, EU286372.1) and *ace2* (AJ868345.1, AJ868312.1)). The three regions were initially aligned individually with ClustalX (Thompson et al., 1997) and thereafter manually adjusted with BioEdit (Hall, 1999) using the similarity criterion (Morrison, 2006). SequenceMatrix (Vaidya et al., 2011) was used to construct a supermatrix. ML analyses were performed using RAxML (Stamatakis et al., 2008) with 500 rapid bootstrap analyses followed by the search of the best-scoring ML tree in one single run. This analysis was done using the facilities offered by the Vital-IT portal in Lausanne, Switzerland (<http://phylobench.vital-it.ch/raxml-bb/>). Classical systematics was used for the second set of data; indeed, the phylogeny of the present taxon was not accurate enough as the overlap between gene alignments across species was too low. Identified molecules were sorted in broad chemical classes and the relation between a particular insect and a particular class of compound was quantified as a percentage of the overall response of this insect to all chemicals identified and having a similar function. Food webs were further analysed using the package “bipartite” (Dormann et al., 2008) in R (R Core Team, 2012). The insect chemotactic orientation towards roots and root acceptance as suitable food by insects were not nested ($p=0.33$ and $p=0.67$, respectively). Nestedness is often related to the robustness of a given ecological network (Bascompte et al., 2003; Bastolla et al., 2009), but a possible explanation why no such networks are observed here can be the very limited amount of data available, mainly focusing on a restricted number of root pests instead of representing the actual complexity of soil food webs. However, the insect

Table 3.1 Chemical cues emitted by plants in the rhizosphere that enable soil-dwelling insects to locate host roots

Insect order	Insect species	Plant specificity	Chemical compound	Class	References
Coleoptera	<i>Hylastes nigrinus</i>	S	α -Pinene	te	Rudinsky (1966) and Rudinsky and Zethner-Møller (1967)
			β -Pinene	te	
			Camphene	te	
	<i>Hylastinus obscurus</i>	S	Pentadecanal	ad	Kamm and Buttery (1984)
			Hexadecanal	ad	
			(<i>E</i>)-2-Hexenal	ad	Tapia et al. (2007)
			Hexanoic acid	ca	Kamm and Buttery (1984)
			Ethyl laurate	es	
			Ethyl benzoate	es	Tapia et al. (2007)
			Methyl benzoate	es	
			Estragole	o	
	<i>Dibrotica v. virgifera</i>	S	(<i>E</i>)- β -Caryophyllene	te	Robert et al. (2012a)
			Oleic acid	li	Hibbard et al. (1994)
			Stearic acid	li	
			DIMBOA	o	Robert et al. (2012b)
	<i>Hyllobius abietis</i>	G	α -Pinene	te	Nordenhem and Nordlander (1994)
			Ethanol	al	

Table 3.1 Chemical cues emitted by plants in the rhizosphere that enable soil-dwelling insects to locate host roots—cont'd

Insect order	Insect species	Plant specificity	Chemical compound	Class	References
	<i>Cylas formicarius</i>	S	Geraniol	al	Wang and Kays (2002)
			Gurjunene	te	
			Humulene	te	
			Ylangene	te	
	<i>Melolontha melolontha</i>	G	Benzaldehyde	ad	Eilers et al. (2012)
			Ethyl acetate	es	
			Limonene	te	
			Linalool	te	
			α -Pinene	Te	
			β -Pinene	te	
			β -Myrcene	te	
			(\pm)-Camphene	te	
			Farnesene	te	
			β -Caryophyllene	te	
			β -Cedrene	te	
			Octanol	al	
Hexyl acetate	es				
	<i>Agriotes</i> spp.	G	Ethyl acetate	es	Morgan and Crumb (1929)
			Nitrobenzene	o	
			Aspartic acid	aa	Thorpe et al. (1946)
			Asparagine	aa	
			Malic acid	ca	
			Succinic acid	ca	
			Glutamine	aa	
			Glutamic acid	aa	

Continued

Table 3.1 Chemical cues emitted by plants in the rhizosphere that enable soil-dwelling insects to locate host roots—cont'd

Insect order	Insect species	Plant specificity	Chemical compound	Class	References		
Diptera	<i>Psila rosae</i>	S	Bornyl acetate	es	Guerin and Ryan (1984) and Ryan and Guerin (1982)		
			2,4-Dimethyl styrene	hy			
			Biphenyl	hy			
			α - and β -Ionone	ke			
			Falcarinol	o	Maki and Ryan (1989) and Maki et al. (1989)		
			Falcarinodiol	o			
			Falcarinodiol monoacetate	o			
			<i>Delia antiqua</i> S	S	<i>n</i> -Propyl disulphide	es	Matsumoto (1970) and Ross and Anderson (1992)
					Methyl disulphide	es	
					Ethyl acetate	es	Mochizuki et al. (1989)
					21 Esters ^a	es	
					<i>n</i> -Propyl mercaptan	mc	
					<i>n</i> -Heptanal	ad	Mochizuki et al. (1989)
					Pentanal	ad	
Hexanal	ad						
Heptanal	ad						
Propanol	al						
Butanol	al						
Pentanol	al						

Table 3.1 Chemical cues emitted by plants in the rhizosphere that enable soil-dwelling insects to locate host roots—cont'd

Insect order	Insect species	Plant specificity	Chemical compound	Class	References
			Hexanol	al	
			Heptanol	al	
			Valeric acid	ca	
			Caproic acid	ca	
			Enanthic acid	ca	
			Allyl isothiocyanate	ie	Ross and Anderson (1992)
			Sulphur compounds ^b	su	Soni and Finch (1979)
			Ethyl sulphide	su	Matsumoto (1970)
			<i>n</i> -Butyl sulphide	su	
			iso-Butyl sulphide	su	
			<i>n</i> -Butyl methyl sulphide	su	
			<i>n</i> -Butyl ethyl sulphide	su	
			Isopentyl sulphide	su	
			Allyl sulphide	su	
			<i>n</i> -Propyl disulphide	su	Ross and Anderson (1992)
			Tetramethylpyrazine	o	Matsumoto (1970)
	<i>Delia floralis</i>	S	Allyl isothiocyanate	ie	Ross and Anderson (1992)
			<i>n</i> -Dipropyl disulphide	su	
			Allyl alcohol	al	
			Methyl eugenol	o	

Continued

Table 3.1 Chemical cues emitted by plants in the rhizosphere that enable soil-dwelling insects to locate host roots—cont'd

Insect order	Insect species	Plant specificity	Chemical compound	Class	References
	<i>Delia radicum</i> S		Isothiocyanates ^c	ie	Finch and Skinner (1974)
			Allyl isothiocyanate	ie	Košťál (1992) and Ross and Anderson (1992)
			Ethyl isothiocyanate	ie	
			Hexanol	al	Košťál (1992)
			<i>cis</i> -3-Hexen-1-ol	al	
			Linalool	al	
			Hexanal	ad	
			<i>n</i> -Dipropyl disulphide	su	Ross and Anderson (1992)
			Allyl alcohol	o	
			Methyl eugenol	o	
	<i>Coquillettidia</i> spp.		Glycerol	al	Sérandour et al. (2008)
			Uracil	py	
			Thymine	py	
			Uridine	nu	
			Thymidine	nu	

^aMochizuki et al. (1989) listed 21 esters (not listed here) that are attractive to *Delia antiqua*.

^bThese compounds reported by Soni and Finch (1979) were either attractive or repellent to *Delia antiqua* depending on the concentration.

^cFinch and Skinner (1974) reported unspecified isothiocyanates attractive to *Delia radicum*.

“Plant specificity” refers to the host range of the insect; (S) specialist (mono- and oligophagous) and (G) generalist (polyphagous). “Class” describes the broad nature of each compound; (aa) amino acids, (ad) aldehydes, (al) alcohols, (ca) carboxylic acids, (es) esters, (hy) hydrocarbons, (ie) isothiocyanates, (ke) ketones, (li) lipids, (mc) mercaptans, (nu) nucleosides, (py) pyrimidines, (su) sulphides, (te) terpenes and (o) others. Links between insects and these classes are further analysed and presented in Fig. 3.2. Adapted from Johnson and Nielsen (2012).

Table 3.2 Chemical cues emitted by plants in the rhizosphere that enable soil-dwelling insects to accept host roots as a suitable source of food

Insect order	Insect species	Plant specificity	Chemical compound	Class	References	
Diptera	<i>Delia floralis</i>	S	Allyl isothiocyanate	ie	Rygg and Sömme (1972)	
			Glucose	sg	Hopkins et al. (1993)	
			Fructose	sg		
	<i>Psila rosae</i>	S	Chlorogenic acid	o	Cole (1985)	
	<i>Delia antiqua</i>	S	Fructose	sg	Honda and Ishikawa (1987) and Mochizuki et al. (1985)	
			Sucrose	sg		
	Coleoptera	<i>Popillia japonica</i>	G	Sucrose	sg	Ladd (1988)
				Maltose	sg	
				Fructose	sg	
Glucose				sg		
Trehalose				sg		
<i>Costelytra zealandica</i>		S	Sucrose	sg	Sutherland (1971)	
			Aspartic acid	aa		Sutherland and Hillier (1974)
			Glutamic acid	aa		
			Serine	aa		
			Ascorbic acid	o		
<i>Acalymma blomorum</i>		S	Cucurbitacins	te	Eben et al. (1997)	
<i>Diabrotica balteata</i>		S	Cucurbitacins	te	Eben et al. (1997)	
<i>D. v. virgifera</i>		S	Glucose	sg	Bernklau and Bjostad (2008)	
			Fructose	sg		
			Sucrose	sg		
	Cucurbitacins		te	Tallamy et al. (2005)		

Continued

Table 3.2 Chemical cues emitted by plants in the rhizosphere that enable soil-dwelling insects to accept host roots as a suitable source of food—cont'd

Insect order	Insect species	Plant specificity	Chemical compound	Class	References
	<i>Diabrotica undecimpunctata</i>	S	Cucurbitacins	te	Eben et al. (1997)
	<i>Heteronychus arator</i>	G	Alanine	aa	Sutherland et al. (1980)
			Aspartic acid	aa	
			Glutamic acid	aa	
			Maltose	sg	Sutherland and Hillier (1976)
			Sucrose	sg	
			Fructose	sg	
			Glucose	sg	
	<i>Sericesthis geminata</i>		Alanine	aa	Wensler and Dudzinski (1972)
			Leucine	aa	
			Isoleucine	aa	
			Sucrose	sg	
			Glucose	sg	
			Maltose	sg	
	<i>Lepidiota negatoria</i>	G	Fructose	sg	Allsopp (1992)
			Mannose	sg	
			Sucrose	sg	
			Raffinose	sg	
			Trehalose	sg	
			Melezitose	sg	
			Cysteine	aa	
			Glutamic acid	aa	
			Histidine mono-hydrochloride	o	

Table 3.2 Chemical cues emitted by plants in the rhizosphere that enable soil-dwelling insects to accept host roots as a suitable source of food—cont'd

Insect order	Insect species	Plant specificity	Chemical compound	Class	References
	<i>Antitrogus parvulus</i>	G	Sucrose	sg	Allsopp (1992)
			Raffinose	sg	
			Tyrosine	aa	
	<i>Agriotes</i> spp.	G	Glucose	sg	Thorpe et al. (1946)
			Sucrose	sg	
			Fructose	sg	
			Galactose	sg	
			Maltose	sg	
			Stachyose	sg	

“Plant specificity” refers to the host range of the insect; (S) specialist (mono- and oligophagous) and (G) generalist (polyphagous). “Class” describes the broad nature of each compound; (aa) amino acids, (ie) isothiocyanates, (sg) sugars, (te) terpenes and (o) others. Links between insects and these classes are further analysed and presented in Fig. 3.3.

Adapted from Johnson and Gregory (2006).

chemotaxic orientation towards roots and root acceptance as suitable food by insects are not random but, as often when nestedness is not observed (Fortuna et al., 2010), appear to have a modular topology, indicating that particular groups of insects were responding to particular groups of chemical classes, therefore reflecting a certain stability (Krause et al., 2003; Teng and McCann, 2004). Modularity was not significant either, but again, these kinds of tests often require a more diverse and documented data set in order to be interpreted correctly. However, strong trends are still present and are discussed in the following sections.

3.2. Plant location

To date, belowground insects have demonstrated positive chemotaxis to over 70 different chemical compounds exuded by healthy or damaged host roots (Table 1). Even though limited to a relatively small number of insect species and chemical classes, the chemotaxic web resulting from the available literature appears highly complex. Indeed, several species from several orders respond to a number of classes of compounds (Fig. 3.2A and B). However,

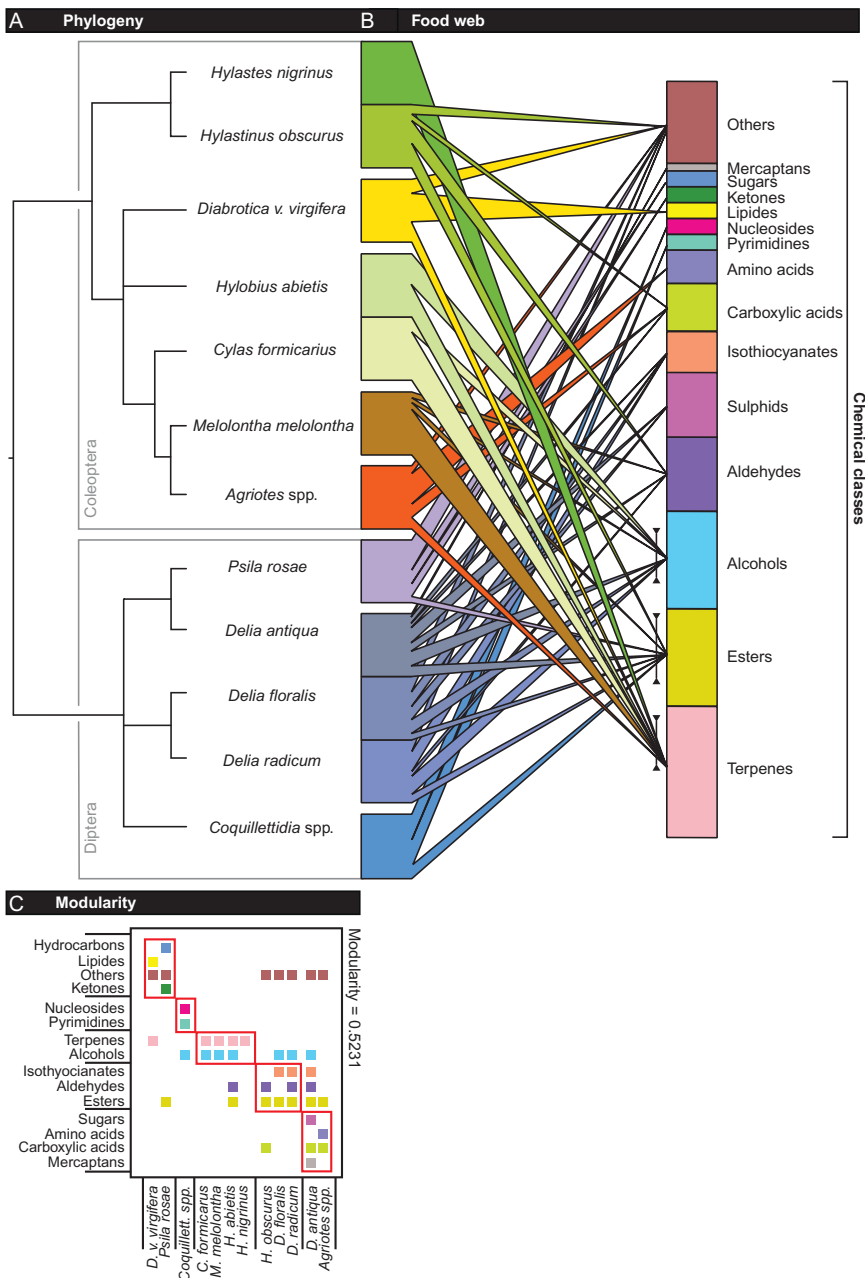


Figure 3.2 Evolution and chemical web of the location of roots by insects. (A) Phylogenetic tree of insects showing positive chemotaxis to certain compounds emitted by roots. (B) Chemical web representing the interactions between each insect species and each chemical class. The width at the origin of the links represents the strength of the relationship as a percentage of the total interaction. Arrows are discussed in the text (Section 3.2). (C) Modular distribution of the insect response to the root-emitted chemicals. Groups are discussed in the text (Section 3.2).

patterns, to some extent supported by the analysis of modularity (Fig. 3.2C), can be highlighted in Fig. 3.2. Indeed, three classes of plant chemical compounds appear to be pivotal in the attraction of insects towards roots and constitute 44% of the chemicals playing such a role. Terpenes are the major class of compounds that insects respond to (17% of total); compounds from this class act as a chemoattractant to insect species in the order Coleoptera, whereas no Dipteran species have been shown to exhibit positive chemotaxis to that same class. Terpenes are volatile organic compounds and act as potent long-distance signals for some foraging insects. Even though their solubility in water is low, moist conditions are necessary for an optimal diffusion of terpenes in soil (Hiltpold and Turlings, 2008), as they may interact significantly more with soil colloids or evaporate when water is lacking. The next two chemical classes with the most documented influence on foraging soil-dwelling insects are esters and alcohols (13.5% of the total, respectively) (Fig. 3.2B). Small esters have relatively high solubility in water and therefore would not be of much help for foraging insects trying to locate roots from a distance but rather support a root acceptance process (see Section 3.3). However, esters' solubility dramatically falls with the length of their carbon chain. As compared to terpenes, the response to esters is more widespread across the current list of soil insect species documented to have interactions with plants based on chemistry (Fig. 3.2B). This general response might be the result of the extensive presence of ester metabolites in plants and roots. Indeed, some major plant hormones belong to this chemical class (Erb et al., 2008; Xie et al., 2013) and several fruit and flower smells perceived as sweet are esters. Equally important as esters, alcohols represent the third main class of chemicals that insects rely on to locate potential root hosts (Fig. 3.2B). Similar to esters, the water solubility of alcohols dramatically drops with the increasing number of carbon atoms (and/or their special organisation) attached to the hydroxyl group. As most of the molecules belonging to this class are rather complex (Table 3.1) and their water solubility is low enough, they can be classified as volatile organic compounds. Whereas these three classes of chemical compounds all diffuse mainly in the subterranean atmosphere and may act as distance cues, several of the remaining classes have various water solubility and can be considered as either water-soluble or not. However, they may still trigger foraging behaviour in certain insects or constitute a component of blends that induce such behaviour. It is also interesting to note that subterranean insects that are considered to have a specific relationship with their host plant are more likely to respond to very special classes of compounds that would be either avoided by generalist feeders

(isothiocyanate; e.g. [Ranger et al. \(2011\)](#)) or considered neutral (pyrimidines and nucleosides; [Sérandour et al. \(2008\)](#)).

Yet, besides CO₂, soil-dwelling insects also rely on some commonly found classes of compounds in order to adopt the biased-random movement defined by [Johnson and Gregory \(2006\)](#). However, they also use more specific compounds such as water-soluble organic compounds or compounds involved in particular root functions (such as defences) to refine their search and target their appropriate host. Most of the molecules used by insects to locate their host are volatile compounds, suggesting that this type of chemicals play a major role in the long-range foraging behaviour of root-herbivore insects.

3.3. Plant acceptance or rejection

Once the insects have found roots in the soil matrix, they again rely on various chemicals to accept them as suitable food. Acting as contact cues, these molecules are likely to be more soluble in water, therefore less motile in soil ([Johnson and Gregory, 2006](#); [Payne and Gregory, 1988](#)), than volatile organic compounds used by insect to locate their potential host root over a longer distance. Johnson and Gregory's hypothesis is supported by peer-reviewed literature where a large majority of the compounds promoting host acceptance or further feeding are molecules that are soluble in water ([Fig. 3.3B](#); sugars 53% and amino acids 12%). Despite the presence of several systematic groups ([Fig. 3.3A](#)), most of the tested insect species rely on either sugars or amino acids (sometimes both) to accept root material as suitable food. Scarabaeid larvae, in particular, use these two classes of compounds as phagostimulants ([Fig. 3.3A](#) and [B](#)). As scarab larvae are rather sedentary in soil, they might have evolved a more accurate method in detecting water-soluble organic compounds as compared to using long-range cues, such as volatile organic compounds. More motile species, such as *Delia* spp. or *Diabrotica* spp., do rely solely on not only water-soluble organic compounds but also less soluble molecules such as terpenes or isothiocyanates as phagostimulants. Because they are longer-range cues, relying on volatile organic compounds as a phagostimulant could allow specialised foraging insects to not only detect the presence of roots but also detect the suitability of the potential food and either to move towards a particular resource or to seek another potent signal. These insects could subsequently use water-soluble organic compounds for shorter-range food acceptance.

Patterns in web structure ([Fig. 3.3C](#)) suggest a strong influence of terpenes on the studied chrysomelid larvae. Indeed, the chrysomelid species

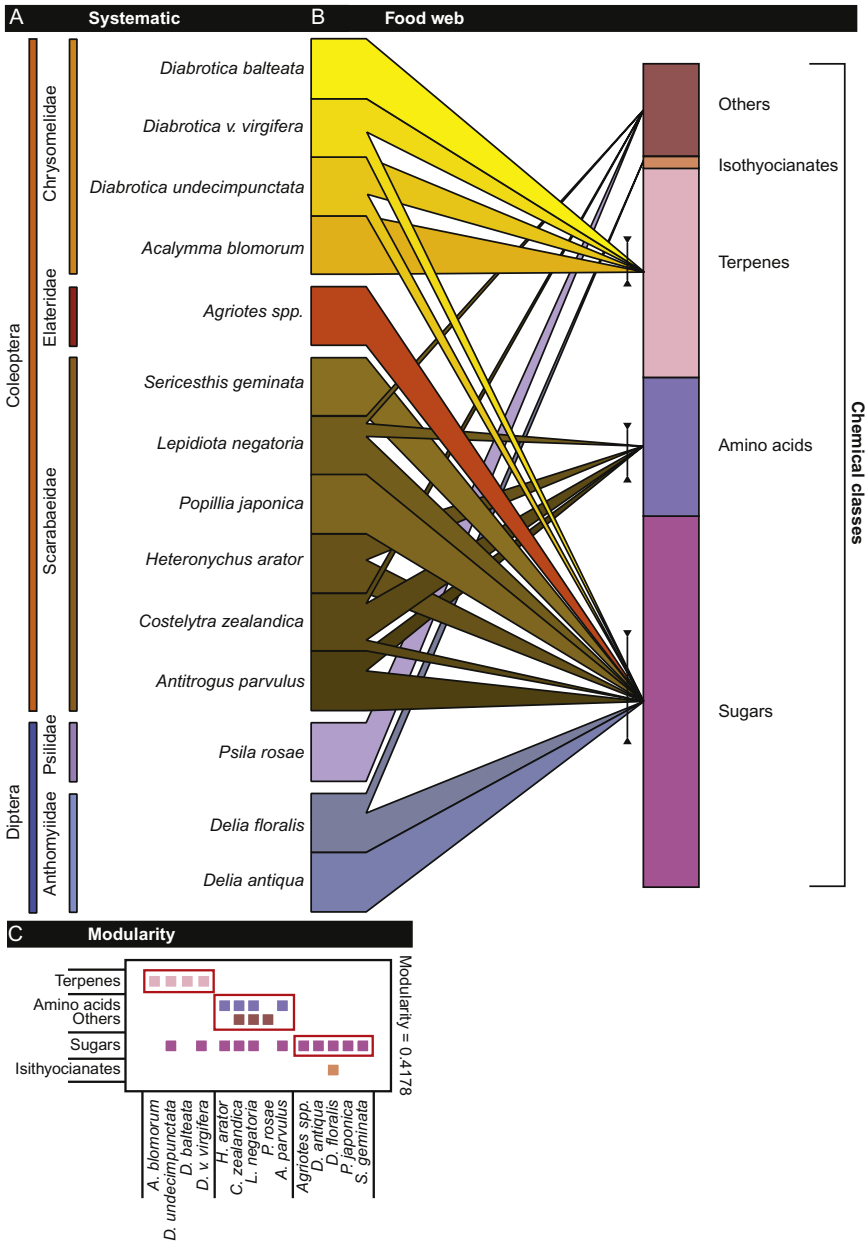


Figure 3.3 Evolution and chemical web of the acceptance of roots by insects. (A) Systematic groups of insect exploiting certain compounds emitted by roots to accept the plant material as suitable food. (B) Chemical web representing the interactions between each insect species and each chemical class. The width at the origin of the links represents the strength of the relationship as a percentage of the total interaction. Arrows are discussed in the text (Section 3.3). (C) Modular distribution of the insect response to the root-emitted chemicals. Groups are discussed in Section 3.3.

Diabrotica spp. and *Acalymma blomorum* Munroe & Smith exhibit a strong link with cucurbitacins (included in the terpene chemical class in the present analysis, Fig. 3.3) (DeHeer and Tallamy, 1991). This surprising affinity to cucurbitacins was discussed by Tallamy et al. (2005) in a comprehensive evolutionary and ecological review. This response is intriguing, especially because the actual main host plants of the larvae of these insect species do not produce these molecules. Cucurbitacins are mainly produced by plants of the Cucurbitaceae family and, because of their bitterness and toxicity, have been thought to be involved in plant protection against insect herbivory (Metcalf, 1985). However, certain insects, such as *Diabrotica* and *Acalymma* species, evolved the ability to sequester this phytochemical in their fat bodies, cuticles, haemolymphs, spermatophores (Andersen et al., 1988; Ferguson and Metcalf, 1985; Tallamy et al., 2000) and interestingly also in developing eggs for further protection of the progeny (Tallamy et al., 1998). When cucurbitacins were sequestered in eggs and larvae, the survival of *Diabrotica undecimpunctata* L. was significantly improved after exposure to the entomopathogenic fungus *Metarhizium anisopliae* (Tallamy et al., 1998). As soil is a pathogen-rich environment, this may explain why females of this insect species allocate almost 80% of their sequestered cucurbitacins into their eggs and in the egg-coating mucus (Tallamy et al., 2000). Notwithstanding that it is likely that cucurbitacins in rootworm are vertically transmitted by parental beetles rather than acquired by the larvae themselves (Tallamy et al., 2005), it is interesting to note that the larval arrestment and feeding behaviour triggered by cucurbitacins in various *Diabroticina* species (Eben et al., 1997) can potentially be useful in rootworm larval management (Hiltbold and Turlings, 2012; Hiltbold et al., 2012).

Recent studies on Collembola, which are expected to obtain carbon and nitrogen from litter, showed that in the presence of roots, these previously assumed decomposers switch from feeding on litter and acquire nutrients from living roots and therefore act as herbivores (Endlweber et al., 2009). Chemical phagostimulants have not yet been identified, but considering the ubiquitousness of Collembola in soil, such switches are likely to impact plants that may actually face much more risk of herbivory than commonly expected in soils.

Insects also rely on exudates to identify unsuitable resources. Interestingly, most of the identified deterrent compounds belong to the isoflavonoid class of molecules (Johnson and Gregory, 2006 and references therein). Isoflavonoids are widely found in several plant groups, particularly in legumes, and are involved in several biological processes in the shoots and the roots.

Interestingly, their solubility in water varies with the number of bonds with sugar residues, making them either local water-soluble organic compound cues or more distal volatile organic compound signals, possibly allowing mobile insects to discriminate (e.g. accept/reject) at a certain distance from the root. While those examples listed by Johnson and Gregory (2006) were mainly repellent, others such as formononetin were actually attractive to larvae of the clover root weevil, *Sitona lepidus* (Coleoptera: Curculionidae) (Johnson et al., 2005).

Several questions on how insects use and respond to root exudates while in the process of accepting or rejecting plant material as suitable food remain unsolved. However, the present discussion highlights that water-soluble organic compounds are crucial for various insect species in the process of host acceptance. While this apparent homogeneity in insect response to sugars and amino acids is unlikely to explain insect specificity to a certain diet, this is important to note that ratios between these chemicals can mediate a specific response (e.g. Bernklau and Bjostad, 2008), therefore allowing insects to specialise on particular plant species. With the realisation that root herbivores use several other classes of compounds as phagostimulants, a broad field of research is now open, an undertaking that is sure to lead knowledge applicable to applied science, especially in the context of plant breeding for natural insect resistance. This could be achieved by selecting plants overexpressing deterrent compounds or roots lacking one or several key phagostimulants (Hiltpold and Turlings, 2012) or in the development of baited soil insecticides (Bernklau and Bjostad, 2005).



4. METHODOLOGY IN STUDYING ROOT-INSECT INTERACTIONS

Developing experimental procedures to study subterranean interactions between plants and insects is challenging and studying insect behaviour without seeing the insect necessitates inventiveness. In the context of interactions mediated by root volatile organic compounds and water-soluble organic compounds, researchers also face the challenge of collecting and isolating chemicals from the soil for identification purposes. Several peer-reviewed papers, book chapters and handbooks describe proven and innovative methods to study the rhizosphere (e.g. Dawson and Byers, 2008; Johnson et al., 2007; Luster and Finlay, 2006; Mankin et al., 2008; Rasmann et al., 2012b). Therefore, in the following sections, we only focus

on some recently published approaches to study root–insect interactions and the chemicals involved in these particular trophic links. Details on methods that are not discussed in the succeeding text were reviewed by [Neumann et al. \(2009\)](#) or described in the publications cited earlier.

4.1. Insect behaviour in soil

4.1.1 *What did the insect feed on?*

This apparently trivial question might be hard to tackle when a new or barely known species is the focus of a research programme. For instance, [Hill and O'Malley \(2010\)](#) conducted a series of biological studies on the Mount Hermon June beetle (*Polyphylla barbata*, Coleoptera: Scarabaeidae). This beetle is endemic to the Zayante Sandhills of the Santa Cruz Mountains in the San Francisco Bay Area (California, USA) and is listed as an endangered species. The species was believed to be specialist on tree roots but very little was known about its host plant range, making the selection of appropriate conservation practices problematic. During a two-year study, [Hill and O'Malley \(2010\)](#) captured beetle larvae and collected their frass. After visual microscopic analyses of the fragments contained in the frass, they could only identify fungal and plant structures ([Hill and O'Malley, 2010](#)). However, the authors described in detail the vegetation in the habitats from which they collected the larvae ([Hill and O'Malley, 2010](#)). Therefore, specific primers could be designed according to the surrounding vegetative composition and more detailed analyses could be conducted. Indeed, molecular tools allow *a priori* and *a posteriori* identification of food sources from digested material and could be a very potent approach in the qualification and quantification of belowground food webs. In an *a priori* approach, [Wallinger et al. \(2012\)](#) designed a diagnostic PCR (polymerase chain reaction) to identify various plant taxa and species from the click beetle (*Agriotes* spp.) gut content or faeces. After having designed primers to amplify specific fragments of chloroplast DNA from the tmL-F region, [Wallinger et al. \(2012\)](#) distinguished plant DNA from the entire insect body DNA and other DNAs from the gut contents. The optimised diagnostic PCR estimated that 30% of *Agriotes* tested positive for DNA of at least one of the 15 targeted plant species ([Wallinger et al., 2012](#)). Using a similar approach, plant material from various species could be detected in the guts of click beetles over a period of 72 h postconsumption ([Wallinger et al., 2013](#)). *Agriotes* species seemed to prefer rhizome- and rosette-forming plants to those species that grew tap or fine roots ([Wallinger et al., 2013](#)). These studies, as well as an extensive

number of others conducted in various ecosystems (e.g. Chapter 5; Campos-Herrera et al., 2011, 2012; Chapman et al., 2012; Garipey and Messing, 2012; Garipey et al., 2008; Lundgren et al., 2009a), support the use of molecular approaches as a powerful tool in describing *a priori* trophic links between known species. In an *a posteriori* approach, next-generation sequencing is a highly potent tool to assess the diet of several animals from various groups (Pompanon et al., 2012 and references therein). This approach seems to be much more consistent and comprehensive than other techniques used so far, such as protein electrophoresis (e.g. Paill et al., 2002; Walrant and Loreau, 1995), the use of antibodies (e.g. Harwood et al., 2001) or stable isotopes (e.g. Sasakawa, 2011; Traugott et al., 2007). High-throughput sequencing results in the sequencing of many of the species' DNA present in the guts of a particular root-insect feeder. Sequences can then be *a posteriori* compared to existing databases and the dietary regime of an animal determined. The costs of such analyses decline as new products and technologies evolve, and together with the ever-expanding publicly available DNA libraries, this approach is very potent when identifying trophic linkages within subterranean food webs. Next-generation sequencing may also be a very powerful tool for understanding how insects adapt to plant direct defences (e.g. Pauchet et al., 2010).

To summarise, molecular tools offer great potential for ecologists to study the considerable complexity of subterranean food webs (also see Chapter 5). In soil, where direct observation is seldom possible, this indirect estimation of the dietary behaviour/preference of insects will certainly provide useful applied information in various contexts such as agroecology, conservation, alien species monitoring or more fundamental knowledge, such as energy flow between/within ecosystem niches.

4.1.2 What is the foraging behaviour of a soil-dwelling pest?

Once the dietary range of a particular insect is described, one could wonder how the insect got to its food source. Again, the lack of an easy direct observation of a subterranean insect foraging for its root makes the description of this biological process difficult. Several methods in laboratory, greenhouse and field setups are well established and often used to address behavioural questions on belowground insects (Dawson and Byers, 2008). Recently, host plant selection by WCR larvae was assessed (Robert et al., 2012a) in a modified belowground olfactometer (Rasmann et al., 2005). Allowed to move in an empty glass tube, the pest larvae were attracted towards roots already infested by conspecifics in addition to performing better in

comparison to larvae fed on uninfested roots (Robert et al., 2012a). While practical, this approach has two major general drawbacks: (1) the insects are moving in an environment far different from their natural niche, lacking any aggregates or particles to crawl on and around, and (2) they may be affected by direct light exposure, which could dramatically disturb their behaviour. In an interesting attempt to solve this first issue, Downie et al. (2012) developed a synthetic transparent, porous and heterogeneous substrate. The water retention of this “transparent” soil was similar to vermiculite and the polymer used absorbed nutrient that would subsequently be available for growing root systems (Downie et al., 2012). Various root growth parameters measured in transparent soil were more similar to those in soil or sand than to those grown in gels, indicating that this synthetic transparent media could accurately mimic natural conditions while allowing direct observation in a complex matrix (Downie et al., 2012). Designing experiments with transparent soil in an environment with low-intensity light would certainly still allow direct observation while reducing the impact of light on the subterranean organisms and on the root physiology. In order to completely isolate the insect from light, there are not many other solutions than using soil itself, which prevents direct observation.

To assess the behaviour of WCR larvae in response to synthetic blends of attractant and feeding stimulants, Hiltpold et al. (2012) designed a destructive laboratory experiment where the insect was monitored by digging out racks previously burrowed in soil microcosms. Visually checking for the presence of larvae in the individual racks, they evaluated the attractiveness of the offered blends of volatile organic compounds and water-soluble organic compounds and further attempted to develop novel biological control strategies (Hiltpold et al., 2012). However, such destructive approaches are not appropriate for long-term observations of a particular process. Therefore, Schumann et al. (2013) designed a more sophisticated experiment to evaluate WCR behaviour. The larvae were allowed to move in a rhizotron (Neumann et al., 2009) thin enough so that their position could be recorded over time. Using GIS (geographic information system) analyses, their behaviour in response to the offered volatiles was mapped (Schumann et al., 2013). Adopting a similar approach, Wilson et al. (1995) built up a large-scale observation chamber dug in the field to examine the effect of pesticides on soil communities.

Recent technological improvements make use of acoustic detection or 3D X-ray reconstruction feasible. For instance, acoustic detection of white grub species (Coleoptera: Scarabaeidae) damaging sugarcane and further

computer processing enabled the identification of the species *Dermolepida albohirtum* individuals and of the members of the genus *Antitrogonus* in the field. Indeed, the grubs showed notably distinctive acoustic patterns of activity, therefore potentially helping in the monitoring effort of these melolonthine pests in Australian sugarcane fields (Mankin et al., 2009). Further development is yet still needed to relate acoustic measures with economic thresholds or to improve bioinformatic analyses (Mankin et al., 2009), but such approaches, if also applicable to other species, could be of significant value, enabling nondestructive sampling and monitoring of the insects in their natural belowground environment. While acoustic methods enable researchers to detect active soil insects, the visualisation of their 3D environment still remains very challenging. Root digging and further imaging have been a widely used approach to quantify root properties (e.g. Pierret et al., 2005; Trachsel et al., 2011). Also known as shovelomics, this technique is still valid to assess particular traits and new bioinformatics models and software are currently available to evaluate root systems in both 2D (e.g. Clark et al., 2013) or in 3D (e.g. Griff et al., 2011). Nevertheless, this destructive approach results in a snapshot of the root phenology in time and, therefore, is very limiting when studies aim to address natural process occurring over longer time periods, like root growth or root colonisation of particular niches in soil. Therefore, other systems have been imagined. For instance, Clark et al. (2011) grew rice (*Oryza sativa* L.) in gellan gum and developed a high-throughput platform for 3D imaging. From 40 2D images of a single root system, their software (RootReader3D) generates a high-resolution 3D reconstruction of a root system that can be further analysed for various root traits such as length, width or (substrate) exploitation (Clark et al., 2011). However, as recently demonstrated by Downie et al. (2012), the material used to grow plants can significantly affect root architecture; therefore, preliminary assessment of architectural parameters should be conducted before using such growth material. Yet, the best material for realistic root measures would again ideally be soil itself. Using X-ray computed tomography (Hounsfield, 1973), pioneer studies could describe several processes occurring around the roots without disturbing the system (Aylmore, 1993; Crestana et al., 1986). Indeed, several studies have used this approach to characterise the behaviour of root-feeding insects in relation to their host plants, albeit in simplified systems (Harrison et al., 1993; Johnson et al., 2004a,b). Since the first use of the X-ray tomography in soil sciences three decades ago, this technology has continuously improved and is now very accurate (Mooney et al., 2012). During several development phases, limits

have been significantly pushed further (Mooney et al., 2012 and references therein) and new instruments are now sensitive enough to detect microscopic variation (down to spatial resolution of 0.5 μm) in soil organisation and root structures (Tracy et al., 2010). This very precise imaging procedure offers various opportunities to unravel complex interactions in the rhizosphere and the ongoing development to visualise much larger portions of soil will definitely scale up the possibilities offered by this technology.

4.2. Collection of water-soluble organic compounds and volatile organic compounds in the rhizosphere

The next question that can be asked is “What triggers this behaviour in subterranean insects?”. As already mentioned in the previous sections, insects are very likely to respond to root exudates, along with other belowground cues. On average, 1000–2200 $\text{kg ha}^{-1} \text{ y}^{-1}$ of organic carbon is exuded by roots (Kuzyakov and Domanski, 2000); it is therefore crucial to be able to sample this enormous fraction of organic compounds and to identify their ecological functions. Root exudates can roughly be sorted in two chemical classes: (1) water-soluble molecules that will be mainly sampled from the water phase in soil (water-soluble organic compounds) and (2) volatile molecules, which are substantially less soluble in water and are more likely to be detected in the soil atmosphere. Rather than two separate groups, root exudates are spread over a continuum of water solubility, according to their chemical properties and the abiotic conditions of a particular soil. Rhizodeposition is defined as all organic compounds released both from healthy root systems and from damaged or senescent roots, whereas root exudates comprise organic compounds released by healthy root systems only (Neumann and Römheld, 2007).

4.2.1 Sampling the water phase in a soil matrix

Since exudation is not homogeneous along a root, the first question to address is where to sample for organic rhizodeposition. This can be achieved using gels (i.e. agar, agarose or polyacrylamide) containing chemical reagents that will stain zones with high exudation activity of a particular class of chemical in a root system. The gels can contain dyes that will react with exudates and change in colour, such as a decolouration of the Al–aluminon complex dye by root exudates (Dinkelaker et al., 1993) or the reduction/oxidation of iron ions (Dinkelaker et al., 1993). pH variations resulting from root exudation can also be visualised while using pH indicators as chemical reagents (Plassard et al., 1999).

Once the optimal location for exudate sampling has been determined, localised sampling techniques can be set up, such as establishing a contact between exudation regions and sorbent materials. Depending on the material used, this sampling can be very generalist or highly selective on a particular category of compounds. Microsuction devices have been used to collect the soil solution for further analyses (Dessureault-Rompré et al., 2006) and have been shown to be consistent in contrasting soil textures (Shen and Hoffland, 2007). Besides an accurate sampling of exudates, microsuction offers the possibility to filter the rhizodeposition and therefore remove most of the microorganisms (Shen and Hoffland, 2007). Indeed, microbes are likely to degrade part of the rhizodeposition while and after sampling, therefore making further chemical analyses inaccurate. Filtration has been shown to significantly reduce microbial degradation (Shen and Hoffland, 2007), as well as formaldehyde (Dessureault-Rompré et al., 2006), even though this treatment has to be carefully realised to prevent contamination of the samples with exogenous chemicals or significant changes in pH. Suction can also be used to sample a larger portion of the soil solution (Neumann et al., 2009). In an elegant *in situ* study, pine tree roots were dug out, carefully washed from remaining soil and inserted into a syringe filled with glass beads (Phillips et al., 2008). A fine mesh was placed in the syringe to prevent the glass beads from clogging the outlet while sampling the exudates with a vacuum pump (Phillips et al., 2008). The collection device was then placed back in the ground. *In situ* spatial and seasonal variations were measured resulting in high exudation at the root tips, which was greater in spring than in fall (Phillips et al., 2008). As with any method, this not only produces experimental artefacts but also underpins the importance of temporal variation in the plant physiology, which should be taken into account while studying such biological processes. It also has the advantage of sampling root exudates without much contamination from microbes present in the rhizosphere.

4.2.2 Sampling volatiles emitted from the roots

This field of research has only recently developed and therefore, methodologies to sample volatiles from the gaseous phase of soil are still scarce. In 2005, Rasmann et al. (2005) isolated and identified volatiles from WCR-damaged corn roots using solid-phase microextraction (SPME) fibre. Briefly, they ground root material in liquid nitrogen and placed the resulting powder in sealed glass vials, from which they collected the volatile organic compounds (Rasmann et al., 2005). SPMEs are made of adsorbent material

fused to a silica fibre and, therefore, allow sampling of volatiles without the use of solvent. Adsorbed molecules can be thermally desorbed in the injection device of a gas chromatography (GC)–mass spectrometer and possibly identified. This method is rapid, easy to set up and, in under some conditions, highly sensitive as SPME fibres can adsorb parts per trillion. A comprehensive book on this volatile sampling approach has been recently published and describes in detail the use of SPME fibres (Pawliszyn, 2011). Although SPME has proven its great potential in sampling root volatiles (Rasmann et al., 2005) and monitoring their diffusion in soil (Hiltbold and Turlings, 2008), the original method was destructive, therefore limiting time-related measures. It has to be noted that the method developed by Rasmann et al. (2005) results in the collection of volatiles within the entire root material and not only compounds exuded by the root system. To overcome this drawback, Ali et al. (2010) subsequently developed an *in vivo* non-destructive method to sample volatile organic compounds of citrus roots induced by the root-herbivore *D. abbreviatus* (Coleoptera: Curculionidae). By vacuuming air from a glass chamber containing citrus plants through an adsorbent trap, they could sample rhizospheric volatiles (Ali et al., 2010). After extraction from the traps, mass-spectrometric (MS) analyses led to the identification of volatiles emitted by induced citrus trees (Ali et al., 2010). In addition to the *in situ* collection, this approach offers the possibility to test the collected volatiles for further bioactivity on soil-dwelling microfauna (Ali et al., 2010, 2011) and to monitor for potential temporal variation in volatile organic compound release. Using the same approach, Hiltbold et al. (2011) could confirm that the volatile organic compounds earlier identified by Rasmann et al. (2005) were indeed emitted from maize roots fed upon by WCR (Hiltbold et al., 2010c; Köllner et al., 2008; Rasmann et al., 2005) and were not artefacts from the sampling method (Hiltbold et al., 2011).

Recently, Ali et al. (2012) developed a probe to sample volatiles emitted by citrus tree roots in the field. Stainless steel probes, coupled to adsorbent traps, were buried in the field 20 cm deep at 1 and 10 m from the tree trunk; soil air was sucked through the traps with a vacuum pump and samples were subsequently extracted in solvent and analysed in GC–MS (Ali et al., 2012). The detection of the most abundant volatile organic compound (pregeijerene; Ali et al., 2010) emitted by damaged citrus roots was still possible 10 m from the source (Ali et al., 2012) underpinning the long range of influence of such exudates *in situ* and their potential impact in shaping biological interaction close and relatively far from the root systems.

Danner et al. (2012) demonstrated the great potential of proton-transfer-reaction mass spectrometry in capturing dynamic processes of root volatile emission. This noninvasive approach allows real-time volatile sampling and is highly sensitive; however, the results from the spectrometry can be difficult to link actual compounds as they provide only masses that could be related to several volatiles (Danner et al., 2012).

4.3. The rise of soil chemical ecology

Recent development in both molecular biology and chemical sampling and analyses will certainly allow rapid progresses in the field of soil chemical ecology and chemically mediated interactions in this environment. All described techniques have not only their limitations but also many advantages. Several new techniques or adaptations of existing approaches are currently developed and soil chemical ecology will certainly become, in a close future, a hub in our global understanding of ecosystems.



5. THE CHEMICAL ECOLOGY OF THE WCR: A CASE STUDY OF A SOIL-DWELLING PEST

The WCR is one of the most studied belowground pests, averaging nearly 50 refereed publications per year recently as documented in SCOPUS and more than 85 publications per year as documented in CAB Direct over the four-year period from 2009 to 2012. It is the most important insect pest of corn in the United States and is also expanding its range in parts of Europe. Both the larvae and adult stages have been relatively well studied, but since it is the larval stage that primarily causes economic damage, the ecology and chemical ecology of this stage have increasingly been studied (Mooser and Hibbard, 2005; Spencer et al., 2009). It is for this reason that we are using this organism as an example.

5.1. Larval studies

The larval stage of the WCR is the economically significant life stage of the insect (Pike et al., 1995). Given the economic importance of this pest (discussed in the preceding text), any new knowledge regarding the insect–host plant interactions is beneficial to corn agriculture. However, chemical and behavioural studies of WCR larvae are challenging because of the nature of the soil environment, in addition to the larvae being rather delicate. Unlike working with aboveground insects, which are generally easier to see and

manipulate, it is difficult to design experiments that give accurate insight into behaviours of soil-dwelling insects (but see [Section 4.1](#)). Additional challenges are encountered when testing root exudates, extracts or other behaviourally active compounds, because it is difficult to evenly distribute such materials in the soil matrix. Experiments are often conducted employing techniques that have previously been used with aboveground larvae or adult insects (but see [Section 4.2](#)). While such studies can provide useful information (behavioural, physiological, toxicological, etc.), the results may not accurately reflect what occurs in the natural soil environment. Despite the challenges, we now have substantial knowledge of the specific host location behaviours of this root-feeding larva and the root infochemicals that elicit those behaviours.

Female WCR beetles lay their eggs in the soil and upon hatching, the burden of host location lies with the neonate larvae. The tiny (2 mm) larvae must travel through the soil to locate the roots of a host plant, a feat that must be accomplished within 24 h of hatching in order to avoid death ([MacDonald and Ellis, 1990](#)). The process of host location by WCR larvae involves three distinct, chemically mediated phases: attraction, host recognition and feeding.

5.2. Attraction

Larvae are initially attracted to corn roots by carbon dioxide (CO₂), which is produced by corn roots in the soil ([Harris and Van Bavel, 1957](#)). It is not surprising that WCR larvae and other soil-dwelling insects use CO₂ as an orientation cue. CO₂, which is a primary metabolite of plants, is a small molecule that is able to diffuse readily through soil-pore spaces and establish a concentration gradient in the soil that can serve as a “trail” back to the roots. Other soil-dwelling insects reported to use CO₂ in host location include the carrot fly larva, *Psila rosae* ([Jones and Coaker, 1977, 1979](#)); the black vine weevil, *Otiorhynchus sulcatus* F. ([Klingler, 1957](#)); cockchafers, *Melolontha* spp. ([Klingler, 1957](#)); wireworms ([Klingler, 1965, 1966](#)), *Agriotes* spp. ([Doane et al., 1975](#); [Klingler, 1957](#)); and subterranean termites, *Reticulitermes* spp. ([Bernklau, 2003](#)). [Table 3.1](#) and [Fig. 3.2](#) summarise the response of soil-dwelling insects to metabolites other than CO₂ (the table and figure are discussed in [Section 3](#)). In early experiments involving an olfactometer, WCR larvae were attracted to CO₂ gas at a low rate of 0.3 ml/h ([Strnad and Bergman, 1987](#)). In a later study, [Bernklau and Bjostad \(1998a\)](#) established a detailed dose–response of neonate larvae to CO₂. This key study employed

a vertical “Y” tube filled with glass beads. This particular bioassay apparatus was designed to accommodate the geotropic tendency of the larvae, and the glass beads simulated large soil particles providing thigmotactic cues that larvae normally encounter in the soil (Bernklau, 1997). This study demonstrated that larvae are attracted to CO₂ concentrations as low as 1.25 mmol/mol (0.125%) but respond most strongly to 0.5% CO₂. The reduced response of larvae to higher concentrations (10% and above) was not due to a lack of attraction, but rather to toxic effects of CO₂. The same body of work also showed that WCR larvae are able to detect differences in CO₂ concentrations as low as 12% (Bernklau and Bjostad, 1998a). The remarkable sensitivity of WCR larvae to very small changes in CO₂ concentration may benefit the insects by enabling them to detect a CO₂ gradient while still some distance from the root of a host plant and then accurately follow the trail back to its source.

The experiments that established the details of attraction to CO₂ were conducted in laboratory bioassays in order to allow accurate gas measurements and, hence, these tests do not accurately reflect what actually happens in the soil environment. Soil atmosphere contains CO₂ from root respiration and respiration of soil-dwelling organisms and CO₂ produced from the breakdown of organic material by microorganisms (Clinton and Vose, 1999; Frank et al., 2002). A portion of soil CO₂ is released into the atmosphere during a natural soil–air exchange of CO₂ and oxygen, and a share of the CO₂ that is then retained in the soil reacts with soil–water to form carbonic acid ($\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3$). The movement and concentration of remaining soil CO₂ is influenced by factors such as soil type and texture, compaction, depth and the amount of organic material in the soil (Brady, 1990). CO₂ concentrations in surface soils range from approximately 0.3% in well-aerated soil to 10% in poorly drained soils (Brady, 1990).

Although only a small-scale representation of the field environment, lab experiments that include the use of a soil medium can provide an indication of the abilities and limitations that WCR larvae might have in the soil. In tub assays, CO₂ concentrations measured in soil within 2 cm of the roots of a small (6-day-old) corn seedling were 0.44%, and levels in the same soil (well-aerated top soil amended with 25% compost) without the corn were 0.2% (Bernklau and Bjostad, 1998a). In this scenario, up to 100% of neonate larvae are able to locate the roots of a single corn plant (V2; Ritchie et al., 1992) within 24 h from a distance of 13 cm (Bernklau and Bjostad, unpublished data). When a smaller germinating corn seed (4 days old) was used in this same setup, the CO₂ concentrations were much lower, measuring 0.13%

near the corn seed and 0.10% 13 cm away, but this 25% gradient was sufficient for more than 80% of larvae to locate the corn within 6 hours (Bernklau, 2003).

Under normal conditions, the larva's ability to detect concentration differences causes them to orient to and then follow the correct gradient produced by the host plant (higher) and to ignore other incorrect CO₂ sources in the soil (lower). This instinctive response to CO₂ can also be used to the detriment of the insect. For example, Bernklau and Bjostad (1998b) reported that larvae are attracted away from corn roots by a higher concentration of CO₂ alone. This effect was first demonstrated in choice tests using a glass bead bioassay. In this test, larvae overwhelmingly chose a 5 mmol/mol (0.5%) concentration of CO₂ alone over a collection of volatiles produced by corn roots (which contained 2 mmol/mol of CO₂ (Bernklau and Bjostad, 1998b)). When the same concept was applied in soil bioassays, larvae crawled away from the roots of a growing corn seed and towards an artificial source of CO₂ alone (Bernklau, 2003; Bernklau et al., 2004). In subsequent tests, larvae were attracted away from corn roots by a variety of CO₂-generating materials placed in the soil, including yeast granules, effervescent tablets and sucrose pellets. These experiments were conducted using small plastic tubs filled with moist soil in which a 2-day-old corn seedling was placed in the soil at one end and the CO₂-generating formulation was placed at the opposite end (13 cm away). When granules comprising yeast and corn syrup (with added corn meal and yeast nutrient agar) proved to be the most consistent treatment, additional trials were conducted with the granules, increasing the size of the container and the size of the corn seedling and varying the placement of the granules and/or the larvae. Two arrangements were particularly effective in preventing larvae from locating corn roots. In the first, the CO₂-generating granules were concentrated in a band in the soil halfway between the larvae and the corn seedling. In the second, the granules were concentrated in a band on one side of the container, the corn seed was placed on the other end, and the larvae were introduced midway between the granules and the corn. One interpretation of these results is that, in these two "attraction" arrangements, larvae were attracted to the CO₂ produced by the band of granules and either stayed in this region or remained in this region long enough that they were then too weak to continue on and reach the corn root. The third and least effective arrangement was an attempt to "disrupt" host location by dispersing the CO₂ granules throughout the entire soil volume. This technique only prevented larvae from locating the corn root when a very large amount of granules (18 g)

were applied to the soil. The fact that most of the larvae were able to locate the corn root despite the lack of a CO₂ gradient (no difference in the CO₂ concentration throughout the soil) suggests that larvae may revert to reliance on other cues for host location when they cannot detect a sufficient CO₂ gradient.

Larvae have previously been reported to be attracted to 6-methoxy-2-benzoxazolinone (Bjostad and Hibbard, 1992) and also to specific long-chain fatty acids when carbon dioxide levels were similar on both sides of the choice (Hibbard et al., 1994) (Table 3.1 and Fig. 3.2B). More recently, when (*E*)- β -caryophyllene or ethylene was added to healthy roots, these plants were more attractive to WCR larvae than healthy roots alone (Robert et al., 2012a). Robert et al. (2012b) conducted a series of studies with maize lines with and without 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and concluded that WCR larvae also utilise this compound for cues to “optimally forage”. Blending chemicals previously identified as attractants or phagostimulants to WCR larvae into a capsule shell, Hiltbold et al. (2012) recorded a similar number of second instar larvae attracted to a maize plant or to capsules over a distance of 25 cm in soil under laboratory conditions.

5.3. Host recognition

The second phase of rootworm host location, which we term “host recognition”, involves a behaviour that is unique to this insect. Strnad and Dunn (1990) first reported that the search behaviour exhibited by larvae after being exposed for a short time (only 5 min) to the roots of corn plants is distinctly different from the search behaviour exhibited after exposure to the roots of a nonhost plant. The response to nonhost roots, which these investigators termed “long-range search behaviour”, is characterised by a rapid rate of crawling in a fairly straight path that incorporates few turns and path crossings. In contrast, the “localised search behaviour” is best described as a “tight-turning” movement because it involves slow, minimal movement, with constant sharp turns and path crossings. When exhibiting tight-turning behaviour, larvae are observed to be constantly, yet slowly, moving their heads back and forth and frequently biting on the neutral substrate (Bernklau et al., 2009; Strnad and Dunn, 1990).

The most unusual aspect of this behaviour is that it does not occur while the insect is on the root surface but rather after the larva has been removed from the root and placed on a neutral surface (untreated filter paper).

The host recognition behaviour of the potato tuber moth larva, *Phthorimaea operculella* Zeller, is somewhat similar in that they reduce their rate of movement, make fewer turns and take more frequent and longer pauses in movement (Varela and Bernays, 1988). However, this behaviour is reported to only occur while the larvae are in contact with an extract from the host plant and does not continue when they are removed from the substrate. This “priming” aspect of the rootworm’s tight-turning behaviour raises the question of whether or not this is indeed a host recognition behaviour. Host recognition by insect larvae more commonly involves behaviours such as biting (Bernays et al., 1997; Eigenbrode and Espelie, 1995), palpating (Chapman and Sword, 1993; Harrison, 1987) or arresting behaviour (Heisswolf et al., 2007), and no other insect larva has been reported to exhibit a specific type of search behaviour after being exposed to the host plant. The nature of this unusual behaviour might best be defined by how it benefits the organism in the soil environment. If a larva that has located a host plant root were to lose contact with the root surface, execution of the localised (tight-turning) search behaviour would keep the insect in the immediate vicinity of the root and would likely result in the larva once again contacting the root surface. On the other hand, a larva that has come into contact with an incorrect (nonhost) root would have a better chance of eventually locating an acceptable host root if it were to exhibit the long-range search behaviour. Based on this logic, as well as the original observations made by Strnad and Dunn (1990), it could be argued that a larva exhibits the tight-turning behaviour because it recognised a root as belonging to a host plant and a larva exhibits the long-range search behaviour because it did not recognise a root as a host.

Work is currently being conducted towards the identification of the host recognition factors. In earlier studies, tight-turning by neonate larvae was elicited using a solvent extract of corn roots, demonstrating that the behavioural cues are chemical in nature (Bernklau et al., 2009). However, subsequent experiments showed that the active compounds are not volatile (Bernklau et al., 2009). Recent studies demonstrated that the host recognition behaviour is elicited by a blend of compounds that includes both polar and nonpolar components. In further tests, tight-turning was elicited when a synthetic blend of four small sugars identified from the polar fraction was combined with the active nonpolar fraction. The sugar blend consists of glucose–fructose–sucrose–myoinositol at 30:8:8:2 mg/ml in the active corn root extract (E. Bernklau, unpublished data). The polar fraction contained many other compounds that are not required for behaviour, including

diacids, amino acids and inorganic compounds. It is possible that these and other compounds have an enhancement effect that has not yet become apparent. While the blend of sugars was shown to be active, it has not yet been determined whether all four sugars are necessary to elicit a larval response, and the activity of the sugars individually has not yet been determined. The active nonpolar fraction consists of large lipids containing fatty acyl groups (Bernklau et al., 2013) and recent chromatographic purification and subsequent chemical analysis of the nonpolar fraction provided evidence that the active component is a large (>700 m/z) glycolipid compound that is present in the corn root at a concentration of approximately 2 mg/g (dry weight) of root (E. Bernklau, unpublished data).

5.4. Feeding behaviour

The third phase of host location behaviour by WCR larvae is intense feeding. When a larva reaches a root in the soil, it takes several “test bites”, and if the root is determined to be an appropriate host, the insect typically begins vigorously feeding immediately (Clark et al., 2006). A specific blend of compounds isolated from corn roots serves as feeding stimulants for WCR larvae (Bernklau and Bjostad, 2008). The feeding stimulant blend consists of 30:4:4 mg/ml glucose–sucrose–fructose, plus at least one free fatty acid (FFA) (either linoleic acid (18:2 FA) or oleic acid (18:1 FA), 0.3 mg/ml). Chemical analysis of the liquid pressed from fresh corn roots revealed a blend of small sugars, amino acids, diacids, FFAs and inorganic compounds. In feeding bioassays, the sugar blend alone elicited feeding by approximately 50% of the larvae tested, but none of the other groups of compounds elicited feeding. The number of larvae feeding and the intensity of feeding were significantly increased ($>90\%$ of larvae feeding) with the addition of one of the FFA (linoleic or oleic acid). FFAs also impact other belowground insect pests (see Chapter 2). Branson (1982) previously reported that a 50% sucrose solution elicited feeding by second instar larvae. Although the blend of 20 amino acids identified in the corn roots did not elicit feeding, it is possible that one or more specific amino acids could enhance feeding on the sugar–fatty acid blend. Amino acids alone (Hollister and Mullin, 1998; Kim and Mullin, 1998), as well as blends of sugars and amino acids (Kim and Mullin, 2007), serve as phagostimulatory cues for adult WCRs, and amino acids are documented as chemosensory cues for several root-feeding insects (Johnson and Gregory, 2006), including grass grub, *Costelytra zealandica* (Sutherland and Hilier, 1974); black beetle, *Heteronychus arator* (Sutherland

et al., 1980); Australian scarab, *Sericesthis geminata* (Wensler and Dudzinski, 1972); and scarabaeid beetle, *Lepidiota negatoria* (Melolonthinae) (Allsopp, 1992).

Although the exact host recognition blend has not yet been identified, results to this point suggest that there is a relationship between the feeding and the host recognition cues for WCR larvae. In previous experiments, the identified feeding stimulant blend did not elicit the host recognition behaviour (Bernklau et al., 2009), but the most recent study does suggest some overlap in the two active blends and it is possible that one or more of the compounds in the feeding stimulant blend are also contained in the host recognition blend.

5.5. Application of behavioural cues in agriculture

Identification of specific larval host location cues may have practical implications in rootworm control. In laboratory bioassays, the synthetic feeding stimulant blend of sugars and FFAs increased the efficacy of thiamethoxam insecticide (Bernklau et al., 2011). When larvae were exposed to treatments for 30 min, the LD₅₀ of thiamethoxam insecticide was 568 pg/ml, but this was decreased to 0.045 pg/ml when the feeding stimulant blend was added, causing a 10,000-fold increase in the efficacy of the insecticide. It might be possible to use feeding stimulants, or even the host recognition cues, to enhance the effectiveness of granular insecticide formulations and provide control of WCR damage with a reduced amount of insecticide. As mentioned earlier, the basic concept has already been proven in bioassays with insecticide/behavioural cue combinations (Bernklau et al., 2011) or with capsules releasing these compounds and luring the pest (Hiltpold et al., 2012), but further work is needed to develop formulations that can be applied in the field that would endure for a period of weeks in the soil environment. A second approach might be to reduce or alter amounts of the behavioural cues contained in corn roots through plant breeding or genetic engineering in order to make the roots less recognisable or less palatable to the larvae and thereby resistant to WCR damage.

5.6. Natural enemies, chemical ecology and pest management

WCR not only is a major pest on maize but also has to face a tremendous number of threats in the ground. As larvae are likely to encounter several

pathogens and predators around the root system, understanding these interactions better can have dramatic effects on rootworm populations and the damage they inflict (Lundgren and Fergen, 2010; Pilz et al., 2009). Just as insects forage for plant roots, so too do soil-dwelling entomopathogens use chemical cues to locate and assess their host. Early researchers in this field observed that entomopathogenic nematodes were attracted to roots of *Thuja occidentalis* L. and *Fragaria × ananassa* ‘Elsanta’ that were damaged by root–insect herbivores, whereas the root or insect alone was not attractive to the nematodes (Boff et al., 2002; van Tol et al., 2001). Entomopathogenic nematodes are obligate parasites of insects that usually kill their host with 24–48 h postinfection (Dillman et al., 2012a). Because they are easy to mass-produce (Ehlers, 2001) and effective against most WCR larval stages (Kurzt et al., 2009), nematodes were thought to be very potent biological control agents against this root pest (Kuhlmann and van der Brugt, 1998; Pilz et al., 2009). It was subsequently shown (Rasmann et al., 2005) that herbivory by WCR larvae on the roots of several maize varieties resulted in root production of sesquiterpene (*E*)- β -caryophyllene; this chemical is highly attractive to the entomopathogenic nematode *Heterorhabditis megidis*. Not all maize varieties produce this compound (Hiltbold et al., 2010c; Köllner et al., 2008; Rasmann et al., 2005), but (*E*)- β -caryophyllene can be restored via genetic engineering of the plant to create genetically modified maize compatible with nematode biocontrol (Degenhardt et al., 2009). This ability of the maize root to call for help when damaged significantly reduces WCR populations in the field, and nematode-attractive plants generally suffered relatively less damage when nematodes were applied to the soil (Degenhardt et al., 2009; Hiltbold et al., 2010a,c; Rasmann et al., 2005). As earlier suggested by Hiltbold et al. (2010a), the strain-specific response of entomopathogenic nematodes to root-emitted volatiles has been confirmed (Laznik and Trdan, 2013), underpinning the necessity to carefully examine the behaviour of the entomopathogenic nematodes before their use in biological control exploiting such tritrophic interactions. Examples of such influence of roots on upper trophic levels remain scarce (but see Ali et al., 2010, 2011, 2012, 2013; Rasmann et al., 2011b), but they illustrate the potential of chemical ecology in the management of subterranean pests such as WCR, especially since entomopathogenic nematodes respond to a wide range of common plant volatile organic compounds (Hallem et al., 2011).

Entomopathogenic nematodes are attracted to nonplant chemical compounds as well. Insect hosts also emit volatiles that recruit several species of

entomopathogenic nematodes (Dillman et al., 2012b). Volatile organic compounds emitted by WCR remain poorly explored but could produce additional chemical attractants useful in nematode biological control programmes (Hiltbold et al., 2010a,b).

Not all chemically mediated signals favour the natural enemies of subterranean herbivores; WCR larvae are protected against predation by a potent haemolymph defence that is at least partially chemical in nature. Arthropod predator communities are abundant and diverse within soil systems and can have important effects on subterranean herbivores. WCR larvae are no exception, and each preimaginal life stage is exposed to its own predator community (Lundgren et al., 2009c). Recent work has revealed that the haemolymph of WCR larvae coagulates quickly on the mouthparts of arthropod predators, causing them to abandon their attack and vigorously clean their mouthparts (Lundgren et al., 2009b). In addition to the physical defensive components of the defence, Lundgren et al. (2010) discovered that there is an ethanol extractable fraction of the haemolymph that is repellent to some predators. The chemistry underlying this defence is actively being pursued, and preliminary results by J.G. Lundgren suggest that hydroxamic acids (DIMBOA, MBOA, BOA, etc.) used in WCR larval foraging behaviour have little bioactivity against predators (Fig. 3.4) or are not found in the rootworm haemolymph. It is notable that the chemical aspect of the defence is separate from the well-established cucurbitacin-derived haemolymph defences of *Diabrotica* species (Tallamy et al., 2005). Research seems to indicate that there is a “dose” component to the defence; first and second instars are apparently undefended against larger predators like some ants, carabid beetles, wolf spiders and crickets (J.G. Lundgren personal observation), but first instars effectively repel predatory mites (Prischmann et al., 2011). WCR is not the only *Diabrotica* sp. to display this type of haemolymph defence; at least four other related species have predator-defended larvae (Wallace and Blum, 1971, J.G. Lundgren, personal observation). To summarise, this haemolymph defence is multifaceted and the physical and chemical facets function with varying effectiveness against the predators of WCR larvae (Lundgren and Fergen, 2010; Lundgren et al., 2009a). This is not to say that WCR larvae cannot be effectively managed using generalist predators. Indeed, research shows that conserving healthy soil arthropod predator communities in agroecosystems can overcome the larval defence of WCR and lead to reduced damage to maize roots (Lundgren and Fergen, 2010, 2011, in press).

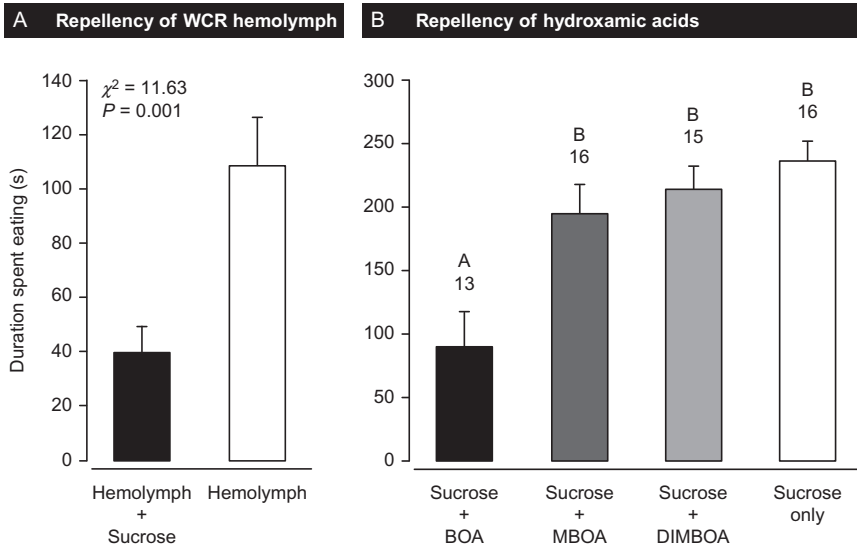


Figure 3.4 Predator response to defensive compounds. (A) Chemical repellency of ethanol-extracted haemolymph of WCR to the predator *Harpalus pensylvanicus* (Coleoptera: Carabidae). (B) Repellency of hydroxamic acids to this WCR predator. Bars represent the mean duration (SEM) spent eating the solutions; above each bar is the number of observation and bars capped with different letters are significantly different from each other (Tukey's test, $\alpha = 0.05$). In 3.4.A, the concentration of haemolymph in the sucrose solution was equivalent to the quantity bled from the third instar WCR. In 3.4.B, predatory beetles were fed 0.24 M sucrose solutions containing 0.01 M concentrations of DIMBOA, MBOA or BOA. Although there was some antipredator bioactivity of BOA, neither this nor any of the other hydroxamic acids could be identified in the haemolymph of WCR.



6. FUTURE CHALLENGES AND CONCLUSIONS

Belowground chemical ecology is a vast but barely explored field of research. The chapter mainly focuses on insects interacting with roots; however, there are hundreds of other types of possible chemical interactions in soil (e.g. [Bonkowski et al., 2009](#); [Rasmann et al., 2012a,b](#)) and therefore challenges in this area of research are tremendous.

Yet, some aspects are likely to receive more attention in the future. As a main research avenue, root defences against herbivores are still mostly unknown, even though progress has been seen recently (e.g. [Johnson et al., 2011](#); [Robert et al., 2012b](#); [Chapter 2](#)). In order to effectively use chemical ecology in pest management strategies, it is crucial to better understand how insects are interacting and coping with root exudates and

chemical defences (comprehensive reviews by [Hiltpold and Turlings, 2012](#); [van Dam, 2009](#)). Given the fact that numerous insect herbivores use plant shoot defensive compounds as host selection cues ([Bernays and Chapman, 1994](#)), it is very likely that root herbivores would do the same and increasing evidence supports this hypothesis (e.g. [Robert et al., 2012a,b](#), but see [Section 5.6](#)). Growing knowledge on the arms race between root defences and root-feeding insect adaptations will surely provide valuable solutions in plant protection strategies.

As with aboveground systems, the effects of global climate change on soil ecosystems are hard to anticipate. Studies are still scarce (but see [Chapter 1](#)); however, elevated CO₂ is unlikely to have direct effect on soil ecosystems ([Staley and Johnson, 2008](#)), especially because average concentration of CO₂ in soils is well above concentrations in the atmosphere ([Payne and Gregory, 1988](#)). Temperature and drought could influence soil porosity by affecting precipitation regimes. Increased porosity significantly enhances the diffusion of volatile organic compounds ([Payne and Gregory, 1988](#)) even though some moisture is necessary for volatile organic compounds to diffuse efficiently in soil ([Hiltpold and Turlings, 2008](#)). Water-soluble organic compound diffusion and availability for rhizospheric organisms are likely to be impaired under dryer conditions. Consequently, subterranean chemical signalling might be affected by global climate change, subsequently impacting several soil-dwelling organisms and ecosystem's functions.

This chapter aims to give an overview of chemical interactions occurring in the rhizosphere between roots, insects and, to some extent, organisms in the upper trophic levels. As primary producers, plants profoundly shape their surrounding environment in many ways. In response, not only insects but also other organisms such as nematodes, bacteria or fungi evolved very complex machinery to perceive and, subsequently, benefit from root chemical exudation of volatile, as long-distance signals, and water-soluble organic compounds, during short-distance foraging or plant acceptance/rejection. Whether beneficial, deleterious or neutral for plants, expanding our knowledge on these interactions is likely to play a major role in future plant protection strategies and will give us tools to guarantee sustainable functionality of this unique and pivotal ecosphere.

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REFERENCES

- Akiyama, K., Matsuzaki, K., Hayashi, H., 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* 435, 824–827.
- Ali, J.G., Alborn, H.T., Stelinski, L.L., 2010. Subterranean herbivore-induced volatiles released by citrus roots upon feeding by *Diaprepes abbreviatus* recruit entomopathogenic nematodes. *J. Chem. Ecol.* 36, 361–368.
- Ali, J.G., Alborn, H.T., Stelinski, L.L., 2011. Constitutive and induced subterranean plant volatiles attract both entomopathogenic and plant parasitic nematodes. *J. Ecol.* 99, 26–35.
- Ali, J.G., Alborn, H.T., Campos-Herrera, R., Kaplan, F., Duncan, L.W., Rodriguez-Saona, C., Koppenhöfer, A.M., Stelinski, L.L., 2012. Subterranean, herbivore-induced plant volatile increases biological control activity of multiple beneficial nematode species in distinct habitats. *PLoS One* 7, e38146.
- Ali, J.G., Campos-Herrera, R., Alborn, H.T., Duncan, L., Stelinski, L.L., 2013. Sending mixed messages: a trophic cascade produced by a belowground herbivore-induced cue. *J. Chem. Ecol.* 39, 1140–1147.
- Allsopp, P.G., 1992. Sugars, amino-acids, and ascorbic acid as phagostimulants for larvae of *Antitrogus parvulus* and *Lepidiota negatoria* (Coleoptera: Scarabaeidae). *J. Econ. Entomol.* 85, 106–111.
- Andersen, J.F., Plattner, R.D., Weisleder, D., 1988. Metabolic transformations of cucurbitacins by *Diabrotica virgifera virgifera* LeConte and *D. undecimpunctata howardi* Barber. *Insect Biochem* 19, 71–78.
- Aylmore, L.A.G., 1993. Use of computer-assisted tomography in studying water movement around plant roots. *Adv. Agron.* 49, 1–54.
- Badri, D.V., Quintana, N., El Kassis, E.G., Kim, H.K., Choi, Y.H., Sugiyama, A., Verpoorte, R., Martinola, E., Manter, D.K., Vivanco, J.M., 2009. An ABC transporter mutation alters root exudation of phytochemicals that provoke an overhaul of natural soil microbiota. *J. Plant Physiol.* 151, 2006–2017.
- Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S., Vivanco, J.M., 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu. Rev. Plant Biol.* 57, 233–266.
- Barber, D.A., Martin, J.K., 1976. The release of organic substances by cereal roots into soil. *New Phytol.* 76, 60–80.
- Bascompte, J., Jordano, P., Melián, C.J., Olesen, J.M., 2003. The nested assembly of plant–animal mutualistic networks. *Proc. Natl. Acad. Sci. U.S.A.* 100, 9383–9387.
- Bastolla, U., Fortuna, M.A., Pascual-García, A., Ferrera, A., Luque, B., Bascompte, J., 2009. The architecture of mutualistic networks minimizes competition and increases biodiversity. *Nature* 458, 1018–1020.
- Bensky, D., Gamble, A., 1986. *Chinese Herbal Medicine: Materia Medica*. Eastland Press, Seattle, USA.
- Bernays, E.A., Chapman, R.F., 1994. *Host-Plant Selection by Phytophagous Insects*. Chapman & Hall, New York.
- Bernays, E.A., Blaney, W.M., Chapman, R.F., Cook, A.G., 1997. The ability of *Locusta migratoria* L. to perceive plant surface waxes. In: Jermy, T. (Ed.), *The Host-Plant in Relation to Insect Behaviour and Reproduction*. Akadémiai Kiadó, Budapest, Hungary, pp. 35–40, Plenum Publishing Corporation New York, NY, USA.

- Bernklau, E.J., 1997. Infochemicals and Host Location by Western Corn Rootworm Larvae. Colorado State University, Fort Collins, CO, USA.
- Bernklau, E.J., 2003. Behavioural Effects of Carbon Dioxide on Western Corn Rootworm and Subterranean Termites with Implications for Pest Management. Colorado State University, Fort Collins, CO, USA, PhD Dissertation.
- Bernklau, E.J., Bjostad, L.B., 1998a. Behavioural responses of first-instar western corn rootworm (Coleoptera: Chrysomelidae) to carbon dioxide in a glass bead bioassay. *J. Econ. Entomol.* 91, 444–456.
- Bernklau, E.J., Bjostad, L.B., 1998b. Re-investigation of host location by western corn rootworm larvae (Coleoptera: Chrysomelidae): CO₂ is the only volatile attractant. *J. Econ. Entomol.* 91, 1331–1340.
- Bernklau, E.J., Bjostad, L.B., 2005. Insecticide enhancement with feeding stimulants in corn for western corn rootworm larvae (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* 98, 1150–1156.
- Bernklau, E.J., Bjostad, L.B., 2008. Identification of feeding stimulants in corn roots for western corn rootworm (Coleoptera: Chrysomelidae) larvae. *J. Econ. Entomol.* 101, 341–351.
- Bernklau, E.J., Fromm, E.A., Bjostad, L.B., 2004. Disruption of host location of western corn rootworm larvae (Coleoptera: Chrysomelidae) with carbon dioxide. *J. Econ. Entomol.* 97, 330–339.
- Bernklau, E.J., Bjostad, L.B., Meihls, L.N., Coudron, T.A., Lim, E., Hibbard, B.E., 2009. Localized search cues in corn roots for western corn rootworm (Coleoptera: Chrysomelidae) larvae. *J. Econ. Entomol.* 102, 558–562.
- Bernklau, E.J., Bjostad, L.B., Hibbard, B.E., 2011. Synthetic feeding stimulants enhance insecticide activity against western corn rootworm larvae, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). *J. Appl. Entomol.* 135, 47–54.
- Bernklau, E.J., Bjostad, L.B., Hibbard, B.E., 2013. Isolation and characterization of host recognition cues in corn roots for larvae of the western corn rootworm (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* Accepted for publication.
- Bjostad, L.B., Hibbard, B.E., 1992. 6-Methoxy-2-benzoxazolinone—a semiochemical for host location by western corn rootworm larvae. *J. Chem. Ecol.* 18, 931–944.
- Blossey, B., Hunt-Joshi, T.R., 2003. Belowground herbivory by insects: influence on plants and aboveground herbivores. *Annu. Rev. Entomol.* 48, 521–547.
- Boff, M.I.C., Van Tol, R., Smits, P.H., 2002. Behavioural response of *Heterorhabditis megidis* towards plant roots and insect larvae. *Biocontrol* 47, 67–83.
- Bonkowski, M., 2004. Protozoa and plant growth: the microbial loop in soil revisited. *New Phytol.* 162, 617–631.
- Bonkowski, M., Villenave, C., Griffiths, B., 2009. Rhizosphere fauna: the functional and structural diversity of intimate interactions of soil fauna with plant roots. *Plant Soil* 321, 213–233.
- Bosak, P.J., Crans, W.J., 2002. The structure and function of the larval siphon and spiracular apparatus of *Coquillettidia perturbans*. *J. Am. Mosq. Control. Assoc.* 18, 280–283.
- Brady, N.C., 1990. *The Nature and Properties of Soils*. MacMillan Publishing, Inc., New York, NY, USA.
- Branson, T.F., 1982. Olfactory response of larvae of *Diabrotica virgifera virgifera* to plant roots. *Entomol. Exp. Appl.* 31, 303–307.
- Britton, E., 1978. A revision of the Australian chafers (Coleoptera: Scarabaeidae: Melolonthinae). Vol. 2. Tribe Melolonthini. *Aust. J. Zool* 26, 1–150, Supplementary Series.
- Brown, V.K., Gange, A.C., 1990. Insect herbivory insect below ground. In: Begon, M., Fitter, A.H., Macfadyen, A. (Eds.), *Advances in Ecological Research*. Elsevier, The Netherlands, pp. 1–58.
- Campos-Herrera, R., El-Borai, F.E., Stuart, R.J., Graham, J.H., Duncan, L.W., 2011. Entomopathogenic nematodes, phoretic *Paenibacillus* spp., and the use of real time

- quantitative PCR to explore soil food webs in Florida citrus groves. *J. Invertebr. Pathol.* 108, 30–39.
- Campos-Herrera, R., El-Borai, F.E., Duncan, L.W., 2012. Real-time PCR as an effective technique to assess the impact of phoresy by *Paenibacillus* sp. bacteria on *Steinernema diaprepesi* nematodes in nature. *Mol. Ecol. Resour.* 12, 885–893.
- Chapman, R.F., Sword, G., 1993. The importance of palpation in food selection by a polyphagous grasshopper (Orthoptera: Acrididae). *J. Insect Behav.* 6, 79–91.
- Chapman, E.G., Schmidt, J.M., Welch, K.D., Harwood, J.D., 2012. Molecular evidence for dietary selectivity and pest suppression potential in an epigeal spider community in winter wheat. *Biol. Control.* 65, 72–86.
- Cipollini, D., Rigsby, C.M., Barto, E.K., 2012. Microbes as targets and mediators of allelopathy in plants. *J. Chem. Ecol.* 38, 714–727.
- Clark, P.L., Vaughn, T.T., Meinke, L.J., Molina-Ochoa, J., Foster, J.E., 2006. *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) larval feeding behaviour on transgenic maize (MON 863) and its isoline. *J. Econ. Entomol.* 99, 722–727.
- Clark, R.T., MacCurdy, R.B., Jung, J.K., Shaff, J.E., McCouch, S.R., Aneshansley, D.J., Kochian, L.V., 2011. Three-dimensional root phenotyping with a novel imaging and software platform. *Plant Physiol.* 156, 455–465.
- Clark, R.T., Famoso, A.N., Zhao, K., Shaff, J.E., Craft, E.J., Bustamante, C.D., McCouch, S.R., Aneshansley, D.J., Kochian, L.V., 2013. High-throughput two-dimensional root system phenotyping platform facilitates genetic analysis of root growth and development. *Plant Cell Environ.* 36, 454–466.
- Clinton, B.D., Vose, J.M., 1999. Fine root respiration in mature eastern white pine (*Pinus strobus*) in situ: the importance of CO₂ in controlled environments. *Tree Physiol.* 19, 475–479.
- Clyne, P.J., Warr, C.G., Freeman, M.R., Lessing, D., Kim, J., Carlson, J.R., 1999. A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in *Drosophila*. *Neuron* 22, 327–338.
- Clyne, P.J., Warr, C.G., Carlson, J.R., 2000. Candidate taste receptors in *Drosophila*. *Science* 287, 1830–1834.
- Cobb, M., 1999. What and how do maggots smell? *Biol. Rev. Camb. Philos.* 74, 425–459.
- Cole, R.A., 1985. Relationship between the concentration of chlorogenic acid in carrot roots and the incidence of carrot fly larval damage. *Ann. Appl. Biol.* 106, 211–217.
- Coleman, D.C., 1976. A review of root production processes and their influence on soil biota in terrestrial ecosystems. In: Andersen, J.M., Macfadyen, A. (Eds.), *The Role of Terrestrial and Aquatic Organisms in Decomposition Processes*. Blackwell, Oxford, UK, pp. 417–434.
- Crestana, S., Casaero, R., Mascarenhas, S., 1986. Using a computer assisted tomography miniscanner in soil science. *Soil Sci.* 142, 56–61.
- Danner, H., Samudrala, D., Cristescu, S.M., Van Dam, N.M., 2012. Tracing hidden herbivores: time-resolved non-invasive analysis of belowground volatiles by proton-transfer-reaction mass spectrometry (PTR-MS). *J. Chem. Ecol.* 38, 785–794.
- Dawson, L.A., Byers, R.A., 2008. Methods for studying root herbivory. In: Johnson, S.N., Murray, P.J. (Eds.), *Root Feeders: An Ecosystem Perspective*. CABI Publishing, Wallingford, UK, pp. 3–19.
- De Bruyne, M., Clyne, P.J., Carlson, J.R., 1999. Odor coding in a model olfactory organ: the *Drosophila* maxillary palp. *J. Neurosci.* 19, 4520–4532.
- De Deyn, G.B., Raaijmakers, C.E., Zoomer, H.R., Berg, M.P., De Ruiter, P.C., Verhoef, H.A., Bezemer, T.M., Van Der Putten, W.H., 2003. Soil invertebrate fauna enhances grassland succession and diversity. *Nature* 422, 711–713.
- De Groot, M., Fäökl, A., Virant-Doberlet, M., 2011. Species identity cues: possibilities for errors during vibrational communication on plant stems. *Behav. Ecol.* 22, 1209–1217.

- De La Peña, E., Echeverría, S.R., Van Der Putten, W.H., Freitas, H., Moens, M., 2006. Mechanism of control of root-feeding nematodes by mycorrhizal fungi in the dune grass *Ammophila arenaria*. *New Phytol.* 169, 829–840.
- Degenhardt, J., Hiltbold, I., Köllner, T.G., Frey, M., Gierl, A., Gershenzon, J., Hibbard, B.E., Ellersieck, M.R., Turlings, T.C.J., 2009. Restoring a maize root signal that attracts insect-killing nematodes to control a major pest. *Proc. Natl. Acad. Sci. U.S.A.* 106, 13213–13218.
- Deheer, C.J., Tallamy, D.W., 1991. Cucumber beetle larval affinity to cucurbitacins. *Environ. Entomol.* 20, 775–788.
- Dekker, T., Geier, M., Carde, R.T., 2005. Carbon dioxide instantly sensitizes female yellow fever mosquitoes to human skin odours. *J. Exp. Biol.* 208, 2963–2972.
- Demathis, F., Kurtz, B., Vidal, S., Smalla, K., 2012. Microbial communities associated with the larval gut and eggs of the western corn rootworm. *PLoS One* 7, e44685.
- Dessureault-Rompré, J., Nowack, B., Schulin, R., Luster, J., 2006. Modified micro suction cup/rhizobox approach for the in-situ detection of organic acids in rhizosphere soil solution. *Plant Soil* 286, 99–107.
- Dethier, V.G., 1947. *Chemical Insect Attractants and Repellents*. Blakiston Co, Philadelphia, PA, USA.
- Dillman, A.R., Chaston, J.M., Adams, B.J., Ciche, T.A., Goodrich-Blair, H., Stock, S.P., Sternberg, P.W., 2012a. An entomopathogenic nematode by any other name. *PLoS Pathog.* 8, e1002527.
- Dillman, A.R., Guillermin, M.L., Lee, J., Kim, B., Sternberg, P.W., Hallem, E.A., 2012b. Olfaction shapes host-parasite interactions in parasitic nematodes. *Proc. Natl. Acad. Sci. U.S.A.* 109, E2324–E2333.
- Dinkelaker, B., Hahn, G., Römheld, V., Wolf, G.A., Marschner, H., 1993. Non-destructive methods for demonstrating chemical changes in the rhizosphere I. Description of methods. *Plant Soil* 155–156, 67–70.
- Dixon, R.A., Strack, D., 2003. Phytochemistry meets genome analysis, and beyond. *Phytochemistry* 62, 815–816.
- Doane, J.F., Klingler, J., 1978. Location of CO₂-receptive sensilla on larvae of wireworms *Agriotes lineatus-obscurus* and *Limonius californicus*. *Ann. Entomol. Soc. Am.* 71, 357–363.
- Doane, J.F., Lee, Y.W., Klingler, J., Westcott, N.D., 1975. The orientation response of *Ctenicera destructor* and other wireworms (Coleoptera: Elateridae) to germinating grain and to carbon dioxide. *Can. Entomol.* 107, 1233–1251.
- Dormann, C.F., Gruber, G., Freund, J., 2008. Introducing the bipartite package: analysing ecological networks. *R news* 8, 8–11.
- Downie, H., Holden, N., Otten, W., Spiers, A.J., Valentine, T.A., Dupuy, L.X., 2012. Transparent soil for imaging the rhizosphere. *PLoS One* 7, e44276.
- Eben, A., Barbercheck, M.E., Aluja, S.M., 1997. Mexican diabroticite beetles: I. Laboratory test on host breadth of *Acalymma* and *Diabrotica* spp. *Entomol. Exp. Appl.* 82, 53–62.
- Ehlers, R.U., 2001. Mass production of entomopathogenic nematodes for plant protection. *Appl. Microbiol. Biotechnol.* 56, 623–633.
- Eigenbrode, S.D., Espelie, K.E., 1995. Effects of plant epicuticular lipids on insect herbivores. *Annu. Rev. Entomol.* 40, 171–194.
- Eilers, E.J., Talarico, G., Hansson, B.S., Hilker, M., Reinecke, A., 2012. Sensing the underground—ultrastructure and function of sensory organs in root-feeding *Melolontha melolontha* (Coleoptera: Scarabaeinae) larvae. *PLoS One* 7, e41357.
- Endlweber, K., Ruess, L., Scheu, S., 2009. Collembola switch diet in presence of plant roots thereby functioning as herbivores. *Soil Biol. Biochem.* 41, 1151–1154.
- Ennis, D.E., Dillon, A.B., Griffin, C.T., 2010. Simulated roots and host feeding enhance infection of subterranean insects by the entomopathogenic nematode *Steinernema carpocapsae*. *J. Invertebr. Pathol.* 103, 140–143.

- Erb, M., Ton, J., Degenhardt, J., Turlings, T.C.J., 2008. Interactions between arthropod-induced aboveground and belowground defenses in plants. *Plant Physiol.* 146, 867–874.
- Erb, M., Glauser, G., Robert, C.A.M., 2012. Induced immunity against belowground insect herbivores—activation of defenses in the absence of a jasmonate burst. *J. Chem. Ecol.* 38, 629–640.
- Eriksson, A., Anfora, G., Lucchi, A., Lanzo, F., Virant-Doberlet, M., Mazzoni, V., 2012. Exploitation of insect vibrational signals reveals a new method of pest management. *PLoS One* 7, e32954.
- Ferguson, J.E., Metcalf, R.L., 1985. Cucurbitacins: plant derived defense compounds for *Diabrotica* (Coleoptera: Chrysomelidae). *J. Chem. Ecol.* 11, 311–318.
- Finch, S., Skinner, G., 1974. Studies of the Cabbage Root Fly. 24th Annual Report for 1973, National Vegetable Research Station, Wellesbourne, Warwick, pp. 84–85.
- Flores, H.E., Vicanco, J.M., Loyola-Vargas, V.M., 1999. ‘Radicle’ biochemistry: the biology of root-specific metabolism. *Trends Plant Sci.* 4, 220–226.
- Fortuna, M.A., Stouffer, D.B., Olesen, J.M., Jordano, P., Mouillot, D., Krasnov, B.R., Poulin, R., Bascompte, J., 2010. Nestedness versus modularity in ecological networks: two sides of the same coin? *J. Anim. Ecol.* 79, 811–817.
- Fraenkel, G.S., 1959. The raison d’être of secondary plant substances. *Science* 129, 1466–1470.
- Frank, A.B., Liebig, M.A., Hanson, J.D., 2002. Soil carbon dioxide fluxes in northern semi-arid grasslands. *Soil Biol. Biochem.* 34, 1235–1241.
- Gagliano, M., 2013. Green symphonies: a call for studies on acoustic communication in plants. *Behav. Ecol.* 24, 789–796.
- Gagliano, M., Mancuso, S., Robert, D., 2012a. Towards understanding plant bioacoustics. *Trends Plant Sci.* 17, 323–325.
- Gagliano, M., Renton, M., Duvdevani, N., Timmins, M., Mancuso, S., 2012b. Acoustic and magnetic communication in plants: is it possible? *Plant Signal. Behav.* 7, 1346–1348.
- Gange, A.C., Brown, V.K., 2002. *Multitrophic Interactions in Terrestrial Systems*. Cambridge University Press, Cambridge, UK.
- Gariepy, T.D., Messing, R.H., 2012. Development and use of molecular diagnostic tools to determine trophic links and interspecific interactions in aphid-parasitoid communities in Hawaii. *Biol. Control.* 60, 26–38.
- Gariepy, T.D., Kuhlmann, U., Gillott, C., Erlandson, M., 2008. Does host plant influence parasitism and parasitoid species composition in *Lygus rugulipennis*? A molecular approach. *B. Entomol. Res.* 98, 217–221.
- Giglio, A., Ferrero, E.A., Perrotta, E., Tripepi, S., Zetto Brandmayr, T., 2003. Ultrastructure and comparative morphology of mouth-part sensilla in ground beetle larvae (insecta, coleoptera, carabidae). *Zool. Anz.* 242, 277–292.
- Glauser, G., Boccard, J., Wolfender, J.L., Rudaz, S., 2013. Metabolomics: application in plant sciences. In: Laemmerhofer, M., Weckewerh, W. (Eds.), *Metabolomics in Practice: Successful Strategies to Generate and Analyze Metabolic Data*. Wiley-VCH, Weinheim, Germany, pp. 311–341.
- Griff, T.E., Novais, J., Bohn, M., 2011. High-throughput phenotyping technology for maize roots. *Biosyst. Eng.* 110, 40–48.
- Grosse-Wilde, E., Kuebler, L.S., Bucks, S., Vogel, H., Wicher, D., Hansson, B.S., 2011. Antennal transcriptome of *Manduca sexta*. *Proc. Natl. Acad. Sci. U.S.A.* 108, 7449–7454.
- Guerin, P.M., Ryan, M.F., 1984. Relationship between root volatiles of some carrot cultivars and their resistance to the carrot fly, *Psila rosae*. *Entomol. Exp. Appl.* 36, 217–224.
- Hall, T.A., 1999. Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids Symp. Ser.* 41, 95–98.

- Hallem, E.A., Dillman, A.R., Hong, A.V., Zhang, Y.J., Yano, J.M., Demarco, S.F., Sternberg, P.W., 2011. A sensory code for host seeking in parasitic nematodes. *Curr. Biol.* 21, 377–383.
- Hansson, B., Stensmyr, M., 2011. Evolution of insect olfaction. *Neuron* 72, 698–711.
- Harris, D.G., Van Bavel, C.H.M., 1957. Root respiration of tobacco, corn and cotton plants. *Agron. J.* 49, 182–184.
- Harrison, G.D., 1987. Host-plant discrimination and evolution of feeding preference in the Colorado potato beetle *Leptinotarsa decemlineata*. *Physiol. Entomol.* 12, 407–415.
- Harrison, R.D., Gardner, W.A., Tollner, W.E., Kinard, D.J., 1993. X-ray computed tomography studies of the burrowing behavior of 4th-instar pecan weevil (Coleoptera, Curculionidae). *J. Econ. Entomol.* 86, 1714–1719.
- Harwood, J.D., Phillips, S.W., Sunderland, K.D., Symondson, W.O.C., 2001. Secondary predation: quantification of food chain errors in an aphid-spider-carabid system using monoclonal antibodies. *Mol. Ecol.* 10, 2049–2057.
- Hedlund, K., Griffiths, B., Christensen, S., Scheu, S., Setälä, H., Tschamntke, T., Verhoef, H., 2004. Trophic interactions in changing landscapes: responses of soil food webs. *Basic Appl. Ecol.* 5, 495–503.
- Heisswolf, A., Gabler, D., Obermaier, E., Muller, C., 2007. Olfactory versus contact cues in host plant recognition of a monophagous Chrysomelid beetle. *J. Insect Behav.* 20, 247–266.
- Hibbard, B.E., Bernklau, E.J., Bjostad, L.B., 1994. Long-chain free fatty-acids: semiochemicals for host location by western corn rootworm larvae. *J. Chem. Ecol.* 20, 3335–3344.
- Hill, K.E., O'Malley, R., 2010. A picky palate? The host plant selection of an endangered June beetle. *J. Insect Conserv.* 14, 277–287.
- Hiltbold, I., Turlings, T.C.J., 2008. Belowground chemical signalling in maize: when simplicity rhymes with efficiency. *J. Chem. Ecol.* 34, 628–635.
- Hiltbold, I., Turlings, T.C.J., 2012. Manipulation of chemically mediated interactions in agricultural soils to enhance the control of crop pests and to improve crop yield. *J. Chem. Ecol.* 38, 641–650.
- Hiltbold, I., Baroni, M., Toepfer, S., Kuhlmann, U., Turlings, T.C.J., 2010a. Selection of entomopathogenic nematodes for enhanced responsiveness to a volatile root signal helps to control a major root pest. *J. Exp. Biol.* 213, 2417–2423.
- Hiltbold, I., Baroni, M., Toepfer, S., Kuhlmann, U., Turlings, T.C.J., 2010b. Selective breeding of entomopathogenic nematodes for enhanced attraction to a root signal did not reduce their establishment or persistence after field release. *Plant Signal. Behav.* 5, 1450–1452.
- Hiltbold, I., Toepfer, S., Kuhlmann, U., Turlings, T.C.J., 2010c. How maize root volatiles influence the efficacy of entomopathogenic nematodes against the western corn rootworm? *Chemoecology* 20, 155–162.
- Hiltbold, I., Erb, M., Robert, C.A.M., Turlings, T.C.J., 2011. Systemic root signalling in a belowground, volatile-mediated tritrophic interaction. *Plant Cell Environ.* 34, 1267–1275.
- Hiltbold, I., Hibbard, B.E., French, D.W., Turlings, T.C.J., 2012. Capsules containing entomopathogenic nematodes as a Trojan horse approach to control the western corn rootworm. *Plant Soil* 358, 11–25.
- Hollister, B., Mullin, C.A., 1998. Behavioural and electrophysiological dose-response relationships in adult western corn rootworm (*Diabrotica virgifera virgifera* LeConte) for host pollen amino acids. *J. Insect Physiol.* 44, 463–470.
- Honda, I., Ishikawa, Y., 1987. Electrophysiological studies on the dorsal and anterior organs of the onion fly larva, *Hylemya antiqua* Meigen (Diptera: Anthomyiidae). *Appl. Entomol. Zool.* 22, 410–416.

- Hopkins, R.J., Griffiths, D.W., Birch, A.N.E., McKinlay, R.G., Hall, J.E., 1993. Relationships between turnip root fly (*Delia floralis*) larval development and the sugar content of swede (*Brassica napus* ssp. *rapifera*) roots. *Ann. Appl. Biol.* 122, 405–415.
- Hounsfield, G.N., 1973. Computerized transverse axial scanning (tomography): I. Description of system. *Brit. J. Radiol.* 46, 1016–1022.
- Hunter, M.D., 2001. Out of sight, out of mind: the impacts of root-feeding insects in natural and managed systems. *Agric. For. Entomol.* 3, 3–9.
- Jepson, W.F., 1937. The morphology of the larva of *Serica brunnea* L. *Bull. Entomol. Res.* 28, 149–165.
- Johnson, S.N., Gregory, P.J., 2006. Chemically-mediated host-plant location and selection by root-feeding insects. *Physiol. Entomol.* 31, 1–13.
- Johnson, S.N., Murray, P.J., 2008. *Root Feeders: An Ecosystem Perspective*. CABI Publishing, Wallingford, UK.
- Johnson, S.N., Nielsen, U.N., 2012. Foraging in the dark—chemically mediated host plant location by belowground insect herbivores. *J. Chem. Ecol.* 38, 604–614.
- Johnson, S.N., Gregory, P.J., Murray, P.J., Zhang, X., Young, I.M., 2004a. Host plant recognition by the root feeding clover weevil, *Sitona lepidus* (Coleoptera: Curculionidae). *Bull. Entomol. Res.* 94, 433–439.
- Johnson, S.N., Read, D.B., Gregory, P.J., 2004b. Tracking larval insect movement within soil using high resolution X-ray microtomography. *Ecol. Entomol.* 29, 117–122.
- Johnson, S.N., Gregory, P.J., Greenham, J.R., Zhang, X.X., Murray, P.J., 2005. Attractive properties of an isoflavonoid found in white clover root nodules on the clover root weevil. *J. Chem. Ecol.* 31, 2223–2229.
- Johnson, S.N., Zhang, X.X., Crawford, J.W., Gregory, P.J., Hix, N.J., Jarvis, S.C., Murray, P.J., Young, I.M., 2006. Effects of carbon dioxide on the searching behaviour of the root-feeding clover weevil *Sitona lepidus* (Coleoptera: Curculionidae). *Bull. Entomol. Res.* 96, 361–366.
- Johnson, S.N., Crawford, J.W., Gregory, P.J., Grinev, D.V., Mankin, R.W., Masters, G.J., Murray, P.J., Wall, D.H., Zhang, X., 2007. Non-invasive techniques for investigating and modelling root-feeding insects in managed and natural systems. *Agric. Forest Entomol.* 9, 39–46.
- Johnson, S.N., Barton, A.T., Clark, K.E., Gregory, P.J., McMenemy, L.S., Hancock, R.D., 2011. Elevated atmospheric carbon dioxide impairs the performance of root-feeding vine weevils by modifying root growth and secondary metabolites. *Glob. Chang. Biol.* 17, 688–695.
- Johnson, S.N., Clark, K.E., Hartley, S.E., Jones, T.H., McKenzie, S.W., Koricheva, J., 2012. Aboveground-belowground herbivore interactions: a meta-analysis. *Ecology* 93, 2208–2215.
- Johnson, S.N., Mitchell, C., McNicol, J.W., Thompson, J., Karley, A.J., 2013. Downstairs drivers—root herbivores shape communities of above-ground herbivores and natural enemies via changes in plant nutrients. *J. Anim. Ecol.* 82, 1021–1030.
- Jones, O.T., Coaker, T.H., 1977. Oriented responses of carrot fly larvae *Psila rosae* to plant odours, carbon dioxide and carrot root volatiles. *Physiol. Entomol.* 2, 189–197.
- Jones, O.T., Coaker, T.H., 1979. Responses of carrot fly larvae, *Psila rosae*, to the odorous and contact-chemostimulatory metabolites of host and non-host plants. *Physiol. Entomol.* 4, 353–360.
- Kamm, J.A., Buttery, R.G., 1984. Root volatile components of red clover identification and bioassay with the clover root borer (Coleoptera, Scolytidae). *Environ. Entomol.* 13, 1427–1430.
- Kaplan, I., Halitschke, R., Kessler, A., Sardanelli, S., Denno, R.F., 2008. Constitutive and induced defenses to herbivory in above- and belowground plant tissues. *Ecology* 89, 392–406.

- Karban, R., 1980. Periodical cicada nymphs impose periodical oak tree wood accumulation. *Nature* 287, 326–327.
- Karban, R., Baldwin, I., 1997. Induced responses to herbivory. University Press of Chicago, Chicago, IL, USA.
- Keil, T.A., 1996. Sensilla on the maxillary palps of *Helicoverpa armigera* caterpillars: in search of the CO₂-receptor. *Tissue Cell* 28, 703–717.
- Kim, J.H., Mullin, C.A., 1998. Structure–phagostimulatory relationships for amino acids in adult western corn rootworm, *Diabrotica virgifera virgifera*. *J. Chem. Ecol.* 24, 1499–1511.
- Kim, J.H., Mullin, C.A., 2007. An isorhamnetin rhamnoglycoside serves as a costimulant for sugars and amino acids in feeding responses of adult western corn rootworms (*Diabrotica virgifera virgifera*) to corn (*Zea mays*) pollen. *J. Chem. Ecol.* 33, 501–512.
- Klinger, J., 1958. Die Bedeutung der Kohlendioxyd-Ausscheidung der Würzeln für die Orientierung der Larven von *Otiorrhynchus sulcatus* F. und anderer bodenbewohnender phytophager Insektenarten. *Mitt. Schweiz. Entomol. Ges.* 31, 205–269.
- Klinger, J., 1957. Über die Bedeutung des Kohlendioxyds für die Orientierung der Larven von *Otiorrhynchus sulcatus* F., *Melolontha* und *Agriotes* (Col.) im Boden (Vorläufige Mitteilung). *Mitt. Schweiz. Entomol. Ges.* 30, 317–322.
- Klinger, J., 1965. On the orientation of plant nematodes and of some other soil animals. *Nematologica* 11, 4–18.
- Klinger, J., 1966. Über den Sitz der CO₂-Rezeptoren bei der Larve von *Otiorrhynchus sulcatus*. *Entomol. Exp. Appl.* 9, 271–277.
- Knochel, D.G., Seastedt, T.R., 2010. Reconciling contradictory findings of herbivore impacts on spotted knapweed (*Centaurea stoebe*) growth and reproduction. *Ecol. Appl.* 20, 1903–1912.
- Kojima, W., Ishikawa, Y., Takanashi, T., 2012. Deceptive vibratory communication: pupae of a beetle exploit the freeze response of larvae to protect themselves. *Biol. Lett.* 8, 717–720.
- Köllner, T.G., Held, M., Lenk, C., Hiltbold, I., Turlings, T.C.J., Gershenzon, J., Degenhardt, J., 2008. A maize (E)-β-caryophyllene synthase implicated in indirect defense responses against herbivores is not expressed in most American maize varieties. *Plant Cell* 20, 482–494.
- Kondoh, Y., Kaneshiro, K.Y., Kimura, K.I., Yamamoto, D., 2003. Evolution of sexual dimorphism in the olfactory brain of Hawaiian *Drosophila*. *Proc. R. Soc. B Biol. Sci.* 270, 1005–1013.
- Košťál, V., 1992. Orientation behavior of newly hatched larvae of the cabbage maggot, *Delia radicum* (L.) (Diptera: Anthomyiidae), to volatile plant metabolites. *J. Insect Behav.* 5, 61–70.
- Krause, A.E., Frank, K.J., Mason, D.M., Ulanowicz, R.E., Taylor, W.W., 2003. Compartments revealed in food web structure. *Nature* 426, 282–285.
- Kuhlmann, U., Van Der Brugt, W.A.C.M., 1998. Possibilities for biological control of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, in Central Europe. *Biocontrol* 19, 59–68.
- Kurzt, B., Hiltbold, I., Turlings, T.C.J., Kuhlmann, U., Toepfer, S., 2009. Comparative susceptibility of larval instars and pupae of the western corn rootworm to infection by three entomopathogenic nematodes. *Biocontrol* 54, 255–262.
- Kuzyakov, Y., Domanski, G., 2000. Carbon input by plants into the soil. *Rev. J. Plant Nutr. Soil Sci.* 163, 421–431.
- Ladd Jr., T.L., 1988. Japanese beetle (Coleoptera: Scarabaeidae): influence of sugars on feeding response of larvae. *J. Econ. Entomol.* 81, 1390–1393.
- Laumann, R.A., Kavčič, A., Moraes, M.C.B., Borges, M., Čokl, A., 2013. Reproductive behaviour and vibratory communication of the neotropical predatory stink bug *Podisus nigrispinus*. *Physiol. Entomol.* 38, 71–80.

- Laznik, Ž., Trdan, S., 2013. An investigation on the chemotactic responses of different entomopathogenic nematode strains to mechanically damaged maize root volatile compounds. *Exp. Parasitol.* 134, 349–355.
- Lu, T., Qiu, Y.T., Wang, G., Kwon, J., Rutzler, M., Kwon, H.W., Pitts, R.J., Van Loon, J.J.A., Takken, W., Carlson, J.R., Zwiebel, L.J., 2007. Odor coding in the maxillary palp of the Malaria vector mosquito *Anopheles gambiae*. *Curr. Biol.* 17, 1533–1544.
- Lundgren, J.G., Fergen, J.K., 2010. The effects of a winter cover crop on *Diabrotica virgifera* (Coleoptera: Chrysomelidae) populations and beneficial arthropod communities in no-till maize. *Environ. Entomol.* 39, 1816–1828.
- Lundgren, J.G., Fergen, J.K., 2011. Enhancing predation of a subterranean insect pest: a conservation benefit of winter vegetation in agroecosystems. *Appl. Soil Ecol.* 51, 9–16.
- Lundgren, J.G., Fergen, J.K., in press. Predator community structure and trophic linkage strength to a focal prey: the influence of the prey's anti-predator defense. *Mol. Ecol.*
- Lundgren, J.G., Ellsbury, M.E., Prischmann, D.A., 2009a. Analysis of the predator community of a subterranean herbivorous insect based on polymerase chain reaction. *Ecol. Appl.* 19, 2157–2166.
- Lundgren, J.G., Haye, T., Toepfer, S., Kuhlmann, U., 2009b. A multifaceted hemolymph defense against predation in *Diabrotica virgifera virgifera* larvae. *Biocontrol Sci. Technol.* 19, 871–880.
- Lundgren, J.G., Nichols, S., Prischmann, D.A., Ellsbury, M.M., 2009c. Seasonal and diel activity patterns of generalist predators associated with *Diabrotica virgifera* immatures (Coleoptera: Chrysomelidae). *Biocontrol Sci. Technol.* 19, 327–333.
- Lundgren, J.G., Toepfer, S., Haye, T., Kuhlmann, U., 2010. Haemolymph defence of an invasive herbivore: its breadth of effectiveness against predators. *J. Appl. Entomol.* 134, 439–448.
- Luster, J., Finlay, R.D., 2006. *Handbook of Methods Used in Rhizosphere Research*. Swiss Federal Research Institute WSL, Birmensdorf.
- Macdonald, P.J., Ellis, C.R., 1990. Survival time of unfed, first-instar western corn rootworm (Coleoptera: Chrysomelidae) and the effects of soil type, moisture, and compaction on their mobility in soil. *Environ. Entomol.* 19, 666–671.
- Maki, A., Ryan, M.F., 1989. Root-mediated effects in carrot resistance to the carrot fly, *Psila rosae*. *J. Chem. Ecol.* 15, 1867–1882.
- Maki, A., Kitajima, J., Abe, F., Stewart, G., Ryan, M.F., 1989. Isolation, identification, and bioassay of chemicals affecting nonpreference carrot-root resistance to carrot-fly larva. *J. Chem. Ecol.* 15, 1883–1897.
- Mankin, R.W., 2012. Applications of acoustics in insect pest management. *CAB Rev.* 7, 1–7.
- Mankin, R.W., Johnson, S.N., Grinev, D.V., Gregory, P.J., 2008. New experimental techniques for studying root herbivores. In: Johnson, S.N., Murray, P.J. (Eds.), *Root Feeders: An Ecosystem Perspective*. CABI Publishing, Wallingford, UK, pp. 20–32.
- Mankin, R.W., Samson, P.R., Chandler, K.J., 2009. Acoustic detection of melolonthine larvae in Australian sugarcane. *J. Econ. Entomol.* 102, 1523–1535.
- Matsumoto, Y., 1970. Volatile organic sulfur compounds as insect attractants with special reference to host selection. In: Wood, D.L., Nakajima, M. (Eds.), *Control of Insect Behavior by Natural Products*. Academic Press, New York, pp. 133–160.
- Merritt, R.W., Dadd, R.H., Walker, E.D., 1992. Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. *Annu. Rev. Entomol.* 37, 349–376.
- Metcalf, R.L., 1985. Plant kairomones and insect pest control. *Bull. Ill. Nat. Hist. Surv.* 35, 175.
- Mochizuki, A., Ishikawa, Y., Matsumoto, Y., 1985. Sugars as phagostimulants for larvae of the onion fly, *Hylemya antiqua* Meigen (Diptera, Anthomyiidae). *Appl. Entomol. Zool.* 20, 465–469.

- Mochizuki, A., Ishikawa, Y., Matsumoto, Y., 1989. Olfactory response of the larvae of the onion fly, *Hylemya antiqua* Meigen (Diptera: Anthomyiidae) to volatile compounds. *Appl. Entomol. Zool.* 24, 29–35.
- Mooser, J., Hibbard, B.E., 2005. A synopsis of the nutritional ecology of larvae and adults of *Diabrotica virgifera virgifera* (LeConte) in the New and Old World—Nouvelle cuisine for the invasive maize pest *Diabrotica virgifera virgifera* in Europe? In: Vidal, S., Kuhlmann, U., Edwards, R. (Eds.), *Western Corn Rootworm: Ecology and Management*. CABI Publishers, Wallingford, pp. 45–61.
- Monroy, F., Van Der Putten, W.H., 2009. Local variation in belowground multitrophic interactions. *Soil Biol. Biochem.* 41, 1689–1695.
- Mooney, S.J., Pridmore, T.P., Helliwell, J., Bennett, M.J., 2012. Developing X-ray computed tomography to non-invasively image 3-D root systems architecture in soil. *Plant Soil* 352, 1–22.
- Morgan, A.C., Crumb, S.E., 1929. Notes on the chemotrophic responses of certain insects. *J. Econ. Entomol.* 21, 913–920.
- Morrison, D.A., 2006. Multiple sequence alignment for phylogenetic purposes. *Aust. Syst. Bot.* 19, 476–539.
- Mukai, H., Hironaka, M., Tojo, S., Nomakuchi, S., 2012. Maternal vibration induces synchronous hatching in a subsocial burrower bug. *Anim. Behav.* 84, 1443–1448.
- Neumann, G., Römheld, V., 2007. The release of root exudates as affected by the plant physiological status. In: Pinton, R., Varanini, Z., Nannipieri, Z. (Eds.), *The Rhizosphere: Biochemistry and Organic Substances at the Soil-Plant Interface*. CRC Press, Boca Raton, pp. 23–72.
- Neumann, G., George, T.S., Plassard, C., 2009. Strategies and methods for studying the rhizosphere—the plant science toolbox. *Plant Soil* 321, 431–456.
- Nordenhem, H., Nordlander, G., 1994. Olfactory oriented migration through soil by root-living *Hyllobius abietis* (L.) larvae (Col, Curculionidae). *J. Appl. Entomol.* 117, 457–462.
- Ortega, Y.K., Pearson, D.E., Waller, L.P., Sturdevant, N.J., Maron, J.L., 2012. Population-level compensation impedes biological control of an invasive forb and indirect release of a native grass. *Ecology* 93, 783–792.
- Otálora-Luna, F., Lapointe, S.L., Dickens, J.C., 2013. Olfactory cues are subordinate to visual stimuli in a neotropical generalist weevil. *PLoS One* 8, e53120.
- Paill, W., Backeljau, T., Grimm, B., Kastberger, G., Kaiser, H., 2002. Isoelectric focusing as a tool to evaluate carabid beetles as predatory agents of the pest slug *Arion lusitanicus*. *Soil Biol. Biochem.* 34, 1333–1342.
- Pauchet, Y., Wilkinson, P., Vogel, H., Nelson, D.R., Reynolds, S.E., Heckel, D.G., Ffrench-Constant, R.H., 2010. Pyrosequencing the *Manduca sexta* larval midgut transcriptome: messages for digestion, detoxification and defence. *Insect Mol. Biol.* 19, 61–75.
- Pawliszyn, J., 2011. *Handbook of Solid Phase Microextraction*. Elsevier, Chennai, India.
- Payne, D., Gregory, P.J., 1988. The soil atmosphere. In: Wild, A. (Ed.), *Russell's Soil Conditions and Plant Growth*. Longman, Harlow, pp. 298–314.
- Phillips, R.P., Erlitz, Y., Bier, R., Bernhardt, E.S., 2008. New approach for capturing soluble root exudates in forest soils. *Funct. Ecol.* 22, 990–999.
- Pichersky, E., Noel, J.P., Dudareva, N., 2006. Biosynthesis of plant volatiles: nature's diversity and ingenuity. *Science* 311, 808–811.
- Pierret, A., Moran, C.J., Doussan, C., 2005. Conventional detection methodology is limiting our ability to understand the roles and functions of fine roots. *New Phytol.* 166, 967–980.
- Pike, D.R., Steffey, K.L., Gray, M.E., Kirby, H.W., Edwards, D.I., Hornbaker, R.H., 1995. *Biological and Economic Assessment of Pesticide Use on Corn and Soybeans*. USDA-National Agricultural Pesticide Impact Assessment Program, Washington, DC.

- Pilz, C., Keller, S., Kuhlmann, U., Toepfer, S., 2009. Comparative efficacy assessment of fungi, nematodes and insecticides to control western corn rootworm larvae in maize. *Biocontrol* 54, 671–684.
- Plassard, C., Meslem, M., Souche, G., Jaillard, B., 1999. Localization and quantification of net fluxes of H⁺ along maize roots by combined use of pH-indicator dye videodensitometry and H⁺-selective microelectrodes. *Plant Soil* 211, 29–39.
- Pompanon, F., Deagle, B.E., Symondson, W.O.C., Brown, D.S., Jarman, S.N., Taberlet, P., 2012. Who is eating what: diet assessment using next generation sequencing. *Mol. Ecol.* 21, 1931–1950.
- Price, P.W., Bouton, C.E., Gross, P., McPherson, B.A., Thompson, J.N., Weis, A.E., 1980. Interactions among three trophic levels—influence of plants on interactions between insect herbivores and natural enemies. *Annu. Rev. Ecol. Syst.* 11, 41–65.
- Prischmann, D.A., Knutson, E.M., Dashiell, K.E., Lundgren, J.G., 2011. Generalist-feeding subterranean mites as potential biological control agents of immature corn rootworms. *Exp. Appl. Acarol.* 55, 233–248.
- Ranger, C.M., Reding, M.E., Oliver, J.B., Schultz, P.B., Moysenko, J.J., Youssef, N., 2011. Comparative efficacy of plant-derived essential oils for managing *Ambrosia* beetles (Coleoptera: Curculionidae: Scolytinae) and their corresponding mass spectral characterization. *J. Econ. Entomol.* 104, 1665–1674.
- Rasmann, S., Agrawal, A.A., 2008. In defense of roots: a research agenda for studying plant resistance to belowground herbivory. *Plant Physiol.* 146, 875–880.
- Rasmann, S., Turlings, T.C.J., 2007. Simultaneous feeding by aboveground and belowground herbivores attenuates plant-mediated attraction of their respective natural enemies. *Ecol. Lett.* 10, 926–936.
- Rasmann, S., Köllner, T.G., Degenhardt, J., Hiltbold, I., Toepfer, S., Kuhlmann, U., Gershenson, J., Turlings, T.C.J., 2005. Recruitment of entomopathogenic nematodes by insect-damaged maize roots. *Nature* 434, 732–737.
- Rasmann, S., Bauerle, T.L., Poveda, K., Vannette, R., 2011a. Predicting root defence against herbivores during succession. *Funct. Ecol.* 25, 368–379.
- Rasmann, S., Erwin, A.C., Halitschke, R., Agrawal, A.A., 2011b. Direct and indirect root defences of milkweed (*Asclepias syriaca*): trophic cascades, trade-offs and novel methods for studying subterranean herbivory. *J. Ecol.* 99, 16–25.
- Rasmann, S., Ali, J.G., Helder, J., Van Der Putten, W.H., 2012a. Ecology and evolution of soil nematode chemotaxis. *J. Chem. Ecol.* 38, 615–628.
- Rasmann, S., Hiltbold, I., Ali, J.G., 2012b. The role of root-produced volatile secondary metabolites in mediating soil interactions. In: Montanaro, G., Dichio, B. (Eds.), *Advances in Selected Plant Physiology Aspects*, 269–290, InTech, Rijeka, Croatia.
- Ritchie, S.W., Hanway, J.J., Benson, G.O., 1992. How a Corn Plant Develops. Iowa State University of Science and Technology Cooperative Extension Service Special Report No. 48
- R Core Team (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, <http://www.r-project.org/>
- Robert, C.A.M., Erb, M., Duployer, M., Zwahlen, C., Doyen, G.R., Turlings, T.C.J., 2012a. Herbivore-induced plant volatiles mediate host selection by a root herbivore. *New Phytol.* 194, 1061–1069.
- Robert, C.A.M., Veyrat, N., Glaser, G., Marti, G., Doyen, G.R., Villard, N., Gaillard, M.D.P., Köllner, T.G., Giron, D., Body, M., Babst, B.A., Ferrieri, R.A., Turlings, T.C.J., Erb, M., 2012b. A specialist root herbivore takes advantage of defensive metabolites to locate nutritious tissues. *Ecol. Lett.* 15, 55–64.
- Ross, K.T.A., Anderson, M., 1992. Larval responses of three vegetable root fly pests of the genus *Delia* (Diptera: Anthomyiidae) to plant volatiles. *B. Entomol. Res.* 82, 393–398.

- Rudinsky, J.A., 1966. Scolytid beetles associated with Douglas fir: response to terpenes. *Science* 152, 218–219.
- Rudinsky, J.A., Zethner-Møller, O., 1967. Olfactory responses of *Hylastes nigrinus* to various host materials. *Can. Entomol.* 99, 911–916.
- Ryan, M.F., Guerin, P.M., 1982. Behavioral responses of the carrot fly larva, *Psila rosae*, to carrot root volatiles. *Physiol. Entomol.* 7, 315–324.
- Rygg, T., Sømme, L., 1972. Oviposition and larval development of *Hylemya floralis* (Fallén) (Diptera, Anthomyiidae) on varieties of swedes and turnips. *Norw. J. Entomol.* 19, 81–90.
- Sasakawa, K., 2011. Laboratory studies on larval food habits of two syntopic, related, granivorous ground beetles *Amara chalcites* and *A. congrua* (Coleoptera: Carabidae): a comparison with stable isotope analysis. *Appl. Entomol. Zool.* 46, 511–518.
- Schillmiller, A.L., Last, R.L., Pichersky, E., 2008. Harnessing plant trichome biochemistry for the production of useful compounds. *Plant J.* 54, 702–711.
- Schneider, D., 1964. Insect Antennae. *Annu. Rev. Entomol.* 9, 103–122.
- Schoonhoven, L.M., Van Loon, J.J.A., Dicke, M., 2005. *Insect-Plant Biology*. Oxford University Press, Oxford.
- Schumann, M., Patel, A., Vidal, S., 2013. Evaluation of an attract and kill strategy for western corn rootworm larvae. *Appl. Soil Ecol.* 64, 178–189.
- Scott, K., Brady Jr., R., Cravchik, A., Morozov, P., Rzhetsky, A., Zuker, C., Axel, R., 2001. A chemosensory gene family encoding candidate gustatory and olfactory receptors in *Drosophila*. *Cell* 104, 661–673.
- Sérandour, J., Reynaud, S., Willison, J., Patouraux, J., Gaude, T., Ravel, P., Lempérière, G., Raveton, M., 2008. Ubiquitous water-soluble molecules in aquatic plant exudates determine specific insect attraction. *PLoS One* 3, e3350.
- Shen, J., Hoffland, E., 2007. In situ sampling of small volumes of soil solution using modified micro-suction cups. *Plant Soil* 292, 161–169.
- Soler, R., Harvey, J.A., Kamp, A.F.D., Vet, L.E.M., Van Der Putten, W.H., Van Dam, N. M., Stuefer, J.F., Gols, R., Hordijk, C.A., Bezemer, T.M., 2007. Root herbivores influence the behaviour of an aboveground parasitoid through changes in plant-volatile signals. *Oikos* 116, 367–376.
- Soni, S.K., Finch, S., 1979. Laboratory evaluation of sulphur-bearing chemicals as attractants for larvae of the onion fly, *Delia antiqua* (Meigen) (Diptera: Anthomyiidae). *Bull. Entomol. Res.* 69, 291–298.
- Spencer, J.L., Hibbard, B.E., Moeser, J., Onstad, D.W., 2009. Behaviour and ecology of the western corn rootworm (*Diabrotica virgifera virgifera* LeConte). *Agric. For. Entomol.* 11, 9–27.
- Staley, J.T., Johnson, S.N., 2008. Climate change impacts on root herbivores. In: Johnson, S.N., Murray, P.J. (Eds.), *Root Feeders: An Ecosystem Perspective*. CABI Publishing, Wallingford, pp. 192–213.
- Stamatakis, A., Hoover, P., Rougemont, J., 2008. A rapid bootstrap algorithm for the RAxML web servers. *Syst. Biol.* 57, 758–771.
- Stange, G., Stowe, S., 1999. Carbon-dioxide sensing structures in terrestrial arthropods. *Microsc. Res. Techniq.* 47, 416–427.
- Steinbrecht, R.A., 1997. Pores structures in insect olfactory sensilla: a review of data and concepts. *Int. J. Insect Morphol.* 26, 229–245.
- Stocker, R.F., 1994. The organization of the chemosensory system in *Drosophila melanogaster*: a review. *Cell Tissue Res.* 275, 3–26.
- Stocker, R.F., 2001. *Drosophila* as a focus in olfactory research: mapping of olfactory sensilla by fine structure, odor specificity, odorant receptor expression, and central connectivity. *Microsc. Res. Techniq.* 55, 284–296.

- Strnad, S.P., Bergman, M.K., 1987. Movement of first-instar western corn rootworms (Coleoptera, Chrysomelidae) in soil. *Environ. Entomol.* 16, 975–978.
- Strnad, S.P., Dunn, P.E., 1990. Host search behavior of neonate western corn rootworm (*Diabrotica virgifera virgifera*). *J. Insect Physiol.* 36, 201–205.
- Strong, D.R., Whipple, A.V., Child, A.L., Dennis, B., 1999. Model selection for a subterranean trophic cascade: root-feeding caterpillars and entomopathogenic nematodes. *Ecology* 80, 2750–2761.
- Sutherland, O.R., 1971. Feeding behaviour of the grass grub *Costelytra zealandica* (White) (Coleoptera: Melolonthinae). 1. The influence of carbohydrates. *N. Z. J. Sci.* 14, 18–23.
- Sutherland, O.R.W., Hillier, J.R., 1974. Olfactory response of *Costelytra zealandica* (Coleoptera: Melolonthinae) to the roots of several pasture plants. *N. Z. J. Sci.* 1, 365–369.
- Sutherland, O.R.W., Hillier, J.R., 1974. Feeding behaviour of the grass grub *Costelytra zealandica* (White) (Coleoptera: Melolonthinae). 3. The influence of amino acids, ascorbic acid, and inorganic salts. *N. Z. J. Zool* 1, 211–216.
- Sutherland, O.R.W., Hillier, J.R., 1976. The influence of maltose and other carbohydrates on the feeding behaviour of *Heteronychus arator* (Scarabaeidae: Coleoptera). *Experientia* 32, 701–702.
- Sutherland, O.R.W., Russell, G.B., Biggs, D.R., Lane, G.A., 1980. Insect feeding deterrent activity of phytoalexin isoflavonoids. *Biochem. Syst. Ecol.* 8, 73–75.
- Swarup, S., Williams, T.I., Anholt, R.R.H., 2011. Functional dissection of odorant binding protein genes in *Drosophila melanogaster*. *Genes Brain Behav.* 10, 648–657.
- Tallamy, D.W., Whittington, D.P., Defurio, F., Fontaine, D.A., Gorski, P.M., Gothro, P., 1998. The effect of sequestered cucurbitacins on the pathogenicity of *Metarhizium anisopliae* (Moniliales: Moniliaceae) on spotted cucumber beetle eggs and larvae (Coleoptera: Chrysomelidae). *Environ. Entomol.* 27, 366–372.
- Tallamy, D.W., Gorski, P.M., Burzon, J.K., 2000. The fate of male-derived cucurbitacins in spotted cucumber beetle females. *J. Chem. Ecol.* 26, 413–427.
- Tallamy, D.W., Hibbard, B.E., Clark, T.L., Gillespie, J.J., 2005. Western corn rootworm, cucurbits and cucurbitacins. In: Vidal, S., Kuhlmann, U., Edwards, R. (Eds.), *Western Corn Rootworm: Ecology and Management*. CABI publishers, Wallingford, pp. 67–93.
- Tapia, T., Perich, F., Pardo, F., Palma, G., Quiroz, A., 2007. Identification of volatiles from differently aged red clover (*Trifolium pratense*) root extracts and behavioural responses of clover root borer (*Hylastinus obscurus*) (Marshall) (Coleoptera: Scolytidae) to them. *Biochem. Syst. Ecol.* 35, 61–67.
- Teng, J., McCann, K.S., 2004. Dynamics of compartmented and reticulate food webs in relation to energetic flow. *Am. Nat.* 164, 85–100.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 25, 4876–4882.
- Thorpe, V.H., Crombie, A.C., Hill, R., Darrah, J.H., 1946. The behaviour of wireworms in response to chemical stimulation. *J. Exp. Biol.* 23, 234–266.
- Tokuda, M., Tanaka, S., Maeno, K., Harano, K.I., Wakamura, S., Yasui, H., Arakaki, N., Akino, T., Fukaya, M., 2010. A two-step mechanism controls the timing of behaviour leading to emergence from soil in adult males of the scarab beetle *Dasylepida ishigakiensis*. *Physiol. Entomol.* 35, 231–239.
- Trachsel, S., Kaeppler, S.M., Brown, K.M., Lynch, J.P., 2011. Shovelomics: high throughput phenotyping of maize (*Zea mays* L.) root architecture in the field. *Plant Soil* 341, 75–87.
- Tracy, S.R., Roberts, J.A., Black, C.R., McNeill, A., Davidson, R., Mooney, S.J., 2010. The X-factor: visualizing undisturbed root architecture in soils using X-ray computed tomography. *J. Exp. Bot.* 61, 311–313.

- Traugott, M., Pázmándi, C., Kaufmann, R., Juen, A., 2007. Evaluating $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$ isotope ratio analysis to investigate trophic relationships of elaterid larvae (Coleoptera: Elateridae). *Soil Biol. Biochem.* 39, 1023–1030.
- Turlings, T.C.J., Hiltbold, I., Rasmann, S., 2012. The importance of root-produced volatiles as foraging cues for entomopathogenic nematodes. *Plant Soil* 359, 51–60.
- Turner, S.L., Li, N., Guda, T., Githure, J., Cardé, R.T., Ray, A., 2011. Ultra-prolonged activation of CO_2 -sensing neurons disorients mosquitoes. *Nature* 474, 87–91.
- Vaidya, G., Lohman, D.J., Meier, R., 2011. SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* 27, 171–180.
- Van Dam, N.M., 2009. Belowground herbivory and plant defenses. *Annu. Rev. Ecol. Evol. Syst.* 40, 373–391.
- Van Dam, N.M., Harvey, J.A., Wackers, F.L., Bezemer, T.M., Van Der Putten, W.H., Vet, L.E.M., 2003. Interactions between aboveground and belowground induced responses against phytophages. *Basic Appl. Ecol.* 4, 63–77.
- Van Dam, N.M., Witjes, L., Svatos, A., 2004. Interactions between aboveground and belowground induction of glucosinolates in two wild *Brassica* species. *New Phytol.* 161, 801–810.
- Van Dam, N.M., Raaijmakers, C.E., Van Der Putten, W.H., 2005. Root herbivory reduces growth and survival of the shoot feeding specialist *Pieris rapae* on *Brassica nigra*. *Entomol. Exp. Appl.* 115, 161–170.
- Van Der Putten, W.H., 2003. Plant defense belowground and spatiotemporal processes in natural vegetation. *Ecology* 84, 2269–2280.
- Van Der Putten, W.H., Bardgett, R.D., De Ruiter, P.C., Hol, W.H.G., Meyer, K.M., Bezemer, T.M., Bradford, M.A., Christensen, S., Eppinga, M.B., Fukami, T., Hemerik, L., Molofsky, J., Schädler, M., Scherber, C., Strauss, S.Y., Vos, M., Wardle, D.A., 2009. Empirical and theoretical challenges in aboveground–belowground ecology. *Oecologia* 161, 1–14.
- Van Tol, R.W.H.M., Van Der Sommen, A.T.C., Boff, M.I.C., Van Bezooijen, J., Sabelis, M.W., Smits, P.H., 2001. Plants protect their roots by alerting the enemies of grubs. *Ecol. Lett.* 4, 292–294.
- Vannette, R.L., Rasmann, S., 2012. Arbuscular mycorrhizal fungi mediate below-ground plant–herbivore interactions: a phylogenetic study. *Funct. Ecol.* 26, 1033–1042.
- Varela, L., Bernays, E.A., 1988. Behavior of newly hatched potato tuber moth larvae, *Phthorimaea operculella* Zell. (Lepidoptera: Gelechiidae), in relation to their host plants. *J. Insect Behav* 1, 261–275.
- Vet, L.E.M., Dicke, M., 1992. Ecology of infochemical use by natural enemies in a tritrophic context. *Annu. Rev. Entomol.* 37, 141–172.
- Vieira, F.G., Sánchez-Gracia, A., Rozas, J., 2007. Comparative genomic analysis of the odorant-binding protein family in 12 *Drosophila* genomes: purifying selection and birth-and-death evolution. *Genome Biol.* 8, R335.
- Vogt, R.G., Riddiford, L.M., 1981. Pheromone binding and inactivation by moth antennae. *Nature* 293, 161–163.
- Vosshall, L.B., Amrein, H., Morozov, P.S., Rzhetsky, A., Axel, R., 1999. A spatial map of olfactory receptor expression in the *Drosophila* antenna. *Cell* 96, 725–736.
- Wallace, J.B., Blum, M.S., 1971. Reflex bleeding: a highly refined defensive mechanism in *Diabrotica* larvae (Coleoptera: Chrysomelidae). *Ann. Entomol. Soc. Am.* 64, 1021–1024.
- Wallinger, C., Juen, A., Staudacher, K., Schallhart, N., Mitterrutzner, E., Steiner, E.M., Thalinger, B., Traugott, M., 2012. Rapid plant identification using species- and group-specific primers targeting chloroplast DNA. *PLoS One* 7, e29473.

- Wallerger, C., Staudacher, K., Schallhart, N., Peter, E., Dresch, P., Juen, A., Traugott, M., 2013. The effect of plant identity and the level of plant decay on molecular gut content analysis in a herbivorous soil insect. *Mol. Ecol. Resour.* 13, 75–83.
- Walrant, A., Loreau, M., 1995. Comparison of iso-enzyme electrophoresis and gut content examination for determining the natural diet of the groundbeetle species *Abax ater* (Coleoptera: Carabidae). *Entomol. Gen.* 19, 253–259.
- Wang, Y., Kays, S.J., 2002. Sweetpotato volatile chemistry in relation to sweetpotato weevil (*Cylas formicarius*) behavior. *J. Am. Soc. Hortic. Sci.* 127, 656–662.
- Weissteiner, S., Huetteroth, W., Kollmann, M., Weissübecker, B., Romani, R., Schachtner, J., Schütz, S., 2012. Cockchafer larvae smell host root scents in soil. *PLoS One* 7, e45827.
- Wenke, K., Kai, M., Piechulla, B., 2010. Belowground volatiles facilitate interactions between plant roots and soil organisms. *Planta* 231, 499–506.
- Wensler, R.J., Dudzinski, A.E., 1972. Gustation of sugars, amino acids and lipids by larvae of the scarabaeid *Sericethis geminata* (Coleoptera). *Entomol. Exp. Appl.* 15, 155–165.
- Wilson, K., Gunn, A., Cherrett, J.M., 1995. The application of a rhizotron to study the subterranean effects of pesticides. *Pedobiologia* 39, 132–143.
- Xie, R.J., Deng, L., Jing, L., He, S.L., Ma, Y.T., Yi, S.L., Zheng, Y.Q., Zheng, L., 2013. Recent advances in molecular events of fruit abscission. *Biol. Plantarum* 57, 201–209.
- Yonekura-Sakakibara, K., Saito, K., 2009. Functional genomics for plant natural product biosynthesis. *Nat. Prod. Rep.* 26, 1466–1487.