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Preface

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This special issue on Soil Chemical Ecology, the 7th in our series, follows previous ones on Allelopathy, 2000; Aquatic Chemical Ecology, 2002; Molecular Chemical Ecology, 2004; Mammalian Chemical Ecology, 2006; Olfactory Ecology, 2008; and Human Impact and Chemical Ecology, 2010. My early discussions with Roxina Soler and Nicole Van Dam morphed into a committee that also included Ted Turlings, Rensen Zeng, and Ann Hagerman. Many others provided helpful suggestions. At the ISCE meeting in Canada in August, 2011, we identified areas that would lend themselves to timely reviews. We were fortunate that essentially all invitees agreed to write. Thus, this issue contains 13 reviews and 2 contributed papers.

The review papers fall more or less into 3 categories that deal with: 1) groups of organisms - bacteria, fungi, nematodes, and soil insects; 2) phenomena that often are better understood in aboveground systems – signaling, foraging, defense, resistance, multifunctional and multitrophic interactions, pheromones, as well as allelopathy; and 3) methodology. The papers are inter-related and cross-referenced in many instances. While no such compendium can be complete, the wide range of organisms and phenomena discussed is remarkable. Aside from serving as a valuable resource for chemical ecologists working in various sub-disciplines, the suggestions made by the authors for focusing future research as well as the need for an emphasis on belowground activities suggest that this is a “garden” expected to “bloom” in coming years.

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Foraging in the Dark – Chemically Mediated Host Plant Location by Belowground Insect Herbivores

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Abstract Root-feeding insects are key components in many terrestrial ecosystems. Like shoot-feeding insect herbivores, they exploit a range of chemical cues to locate host plants. Respiratory emissions of carbon dioxide (CO_2) from the roots is widely reported as the main attractant, however, there is conflicting evidence about its exact role. CO_2 may act as a ‘search trigger’ causing insects to search more intensively for more host specific signals, or the plant may ‘mask’ CO_2 emissions with other root volatiles thus avoiding detection. At least 74 other compounds elicit behavioral responses in root-feeding insects, with the majority (>80 %) causing attraction. Low molecular weight compounds (e.g., alcohols, esters, and aldehydes) underpin attraction, whereas hydrocarbons tend to have repellent properties. A range of compounds act as phagostimulants (e.g., sugars) once insects feed on roots, whereas secondary metabolites often deter feeding. In contrast, some secondary metabolites usually regarded as plant defenses (e.g., dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA)), can be exploited by some root-feeding insects for host location. Insects share several host location cues with plant parasitic nematodes (CO_2, DIMBOA, glutamic acid), but some compounds (e.g., cucurbitacin A) repel nematodes while acting as phagostimulants to insects. Moreover, insect and nematode herbivory can induce exudation of compounds that may be mutually beneficial, suggesting potentially significant interactions between the two groups of herbivores. While a range of plant-derived chemicals can affect the behavior of root-feeding insects, little attempt has been made to exploit these in pest management, though this may become a more viable option with diminishing control options.

Keywords Insect · Nematode · Root exudates · Root-feeders · Soil · Pests

Herbivores in the Soil

Soil-dwelling herbivores comprise mammals (e.g., rodents) and invertebrates (e.g., insects and nematodes), which feed on a wide range of plant species and belowground plant structures (Andersen, 1987; Hunter, 2001; Johnson and Murray, 2008). While root-feeding insects are generally less well-studied than shoot herbivores (Hunter, 2001), it is widely recognized that they play pivotal roles in terrestrial ecosystems (Johnson and Murray, 2008). In agricultural systems, damage caused by belowground herbivores can be profound (Blackshaw and Kerry, 2008), with yield reductions of up to 60 % reported for the vine weevil (*Otiorhynchus sulcatus*) for example (Clark et al., 2012). Similarly, the ecological significance of soil-dwelling herbivores is apparent, with many studies illustrating how they can influence the community dynamics of plants (e.g., De Deyn et al., 2003), soil micro-organisms (e.g., Grayston et al., 2001), and aboveground herbivores (e.g., Johnson et al., 2009). In the context of abundance, it has been shown that root-xylem feeding cicadas in eastern deciduous forest of North America have the greatest collective biomass of any terrestrial animal in terms of biomass per unit area (Karban, 1980). Even in low diversity Australian pastures, it is common for the weight of

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sheep per acre to be exceeded by the weight of root-feeding insects in the soil (Britton, 1978). In this review, we are principally concerned with insect herbivores (see Rasmann et al., 2012, this issue, for discussion of nematodes), although we consider potential similarities and interactions between the two types of invertebrate herbivores.

Aims and Scope

Despite the importance of soil-dwelling herbivores, our understanding of the chemical ecology underpinning their interactions with host plants is limited compared to those herbivores feeding on aerial parts of the plant. In the first attempt to systematically address how root-feeding insects locate and select host plants by using chemicals in the rhizosphere and in root tissues, Johnson and Gregory (2006) collated information from 78 studies. In the current review, we aim to update this synthesis but more importantly identify new issues in the chemical ecology underpinning root location by soil-dwelling insects. Like the article by Johnson and Gregory (2006), we place some terms in inverted commas (e.g., 'attractant') because not all of the studies use the strict definition of the word. We focus on insect herbivores, since the chemical ecology of nematodes is covered by Rasmann et al. (2012, this issue), and we are unaware of any studies that address the chemical ecology of root herbivory by mammals.

Mechanisms of Root Location

The earliest studies addressing insect orientation to roots suggested that they encountered roots at random, and there was little scope for chemical mediation (Lees, 1943; Thorpe et al., 1946). Even though wireworms were 'attracted' to components of plant roots, Thorpe et al. (1946) argued that any orientation to roots was in response to changes in soil architecture (e.g., cracks) in the rhizosphere caused by root growth. Subsequent studies (Klingler, 1957, 1958; Doane et al., 1975) showed that wireworms orientated towards roots using respiratory emissions of carbon dioxide (CO_2), and there has been growing evidence that root exudates are used by a range of herbivores to locate suitable host plants ever since (Bais et al., 2006; Johnson and Gregory, 2006; Wenke et al., 2010). In some cases, it has been established that volatile cues mediate 'attraction' to roots without necessarily identifying the chemical compounds involved. These include location by wireworms (Calkins et al., 1967; Horton and Landolt, 2002), the clover root weevil *Sitona hispidulus* (Wolfson, 1987), the wheat bulb fly (*Delia coarctata*) (Stokes, 1956; Long, 1958; Scott, 1974), and the grass grub (*Costelytra zealandica*) (Sutherland, 1972; Sutherland and Hillier, 1974b).

Respiratory Emissions of Carbon Dioxide

Respiratory emissions of carbon dioxide (CO_2) remains the most widely reported root exudate implicated in the 'attraction' of a number of root herbivores (Table 1). CO_2 is the most abundant gaseous exudate from roots and diffuses relatively rapidly in soil (Payne and Gregory, 1988). Several studies have reported that soil-dwelling insects are sensitive to even very small increases in CO_2 concentrations; 0.02 mmol mol^{-1} for the wireworm *Ctenicera destructor* (Doane et al., 1975) and 0.03 mmol mol^{-1} for the vine weevil, *O. sulcatus* (Klingler, 1958). Conversely, very high concentrations of CO_2 can 'repel' (Klingler, 1958) or become toxic to insects (Bernklau and Bjostad, 1998a). The ubiquitous nature of CO_2, the stronger vertical gradients (between the air and the upper soil), and the high density of roots (>1 cm cm^{-3}) (Gregory, 2006), led Johnson and Gregory (2006) to question whether this was an effective means for root-feeding insects to locate roots, particularly in mixed plant communities and when the herbivore specializes on particular plant species. Instead, they proposed that in some systems CO_2 might act as a 'search trigger' causing insects to forage more intensely within a potential resource patch. This has since been supported empirically for the clover root weevil, *Sitona lepidus* (Johnson et al., 2006) and the cabbage root fly, *Delia radicum* (pers. obs.). Similarly, the European cockchafer, *Melolontha melolontha*, orientated within CO_2 gradients to the source of the CO_2, but this orientation disappeared when other plant-derived signals were present (Reinecke et al., 2008). Beyond this example, we have little understanding of how other root exudates (see section below) interact with respiratory emissions of CO_2, but it seems highly likely that insect behavior will be moderated by the interplay of different signals. In any case, these studies lend support to the idea that CO_2 emissions are supplemented by other chemical signals that may 'attract', 'deter', or even mask (proposed by Reinecke et al., 2008), any attraction to CO_2 sources (Johnson et al., 2006).

Root Exudates Other than CO_2

In their review, Johnson and Gregory (2006) listed around 60 compounds that potentially play a role in host plant location, revised and updated in Table 2. There are several additions, with approximately 74 different compounds now reported in the literature. The vast majority (>80 %) are regarded as 'attractants,' with the remaining compounds having either 'repellent' properties, or being both 'attractive' or 'repellent' depending on concentration. There is a trend for low molecular weight compounds (e.g., alcohols, esters, and aldehydes) to have 'attractant' properties, while hydrocarbons tend to be 'repellent'. As noted by Johnson and Gregory (2006), only methyl eugenol and allyl-isothiocyanate were 'attractive' to more than two insect species (Table 2).

Table 1 Soil insect herbivores showing behavioral responses to CO_2, adapted from Johnson and Gregory (2006) with superscript numbers referring to adjacent references

Insect order	Insect species	Plant specificity	Dose–response	References
Diptera	Carrot root fly *Psilae rosae*	*s*	*dr*[1] *nm*[2]	(von Städler, 1971 [2]; Jones and Coaker, 1977, 1979) [1]
	Cabbage root fly *Delia brassicae*	*s*	*na*	Jones and James (unpublished) cited in Jones and Coaker (1978)
Lepidoptera	Lesser cornstalk borer *Elasmopalpus lignosellus*	*s*	*dr*	(Huang and Mack, 2001, 2002)
Coleoptera	Western corn rootworm *Diabrotica virgifera virgifera*	*s*	*dr*[3] *nm*[4]	(Strnad et al., 1986) [3] (Strnad and Bergman, 1987a) [4] (Macdonald and Ellis, 1990) [4] (Macdonald and Ellis, 1990) [4] (Bernklau and Bjostad, 1998b, a) [2,3]
	Grass grub *Costelytra zealandica*	*s*	*nm*	(Galbreath, 1988)
	Black vine weevil *Otiorhynchus sulcatus*	*g*	*nm*	(Klingler, 1957, 1958, 1965, 1966)
	Wireworms *Agriotes* spp.	*g*	*nm*	(Thorpe et al., 1946; Klingler, 1957, 1958, 1965, 1966)
	Ctenicera destructor	*g*	*nm*	(Doane et al., 1975)
	Agriotes obscurus lineatus	*g*	*nm*	(Doane et al., 1975)
	Limonius californicus	*g*	*nm*	(Doane et al., 1975)
	Hypolithus bicolor	*g*	*nm*	(Doane et al., 1975)
	Cockchafer *Melolontha vulgaris*	*g*	*nm*	(Klingler, 1957)
	European cockchafer *Melontha vulgaris*	*g*	*na*	(Reinecke et al., 2008)
	Southern corn rootworm *Diabrotica undecimpunctata*	*s*	*nm*	(Jewett and Bjostad, 1996)
	Ground beetle *Evarthrus sodalis*	*g*	*dr*	(Hamilton, 1917)
	Clover root weevil *Sitona lepidus*	*s*	*dr*	(Johnson et al., 2006)

Plant specificity refers to the host-plant range of the insects; *g* = generalist feeders (polyphagous), *s* = specialist feeders (mono/oligophagous). Dose response refers to whether insects showed a dose-dependent response to CO_2; *dr* = dose dependent, *nm* = not measured and *na* = information not available

Some studies (e.g., Finch and Skinner, 1974; Soni and Finch, 1979; Mochizuki et al., 1989; Weissteiner and Schutz, 2006) report chemical groups rather than specific compounds, so further breakdown of trends and patterns is difficult to achieve accurately.

The recent inclusion of 1,4-benzoxazin-3-one derivatives (Robert et al., 2012) and formononetin (Johnson et al., 2005) as being ‘attractive’ to western corn rootworm and the clover root weevil, respectively, is interesting as these compounds are usually regarded as having defensive properties in the plant. 2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), in particular, is regarded as having powerful insecticidal properties. Workers have found it challenging to identify chemicals underpinning host plant ‘attraction’ by the western corn rootworm; initial findings (Bjostad and Hibbard, 1992; Hibbard et al., 1994) were later revised (Bernklau and Bjostad, 1998b) or found to conflict with other studies (Xie et al., 1990, 1992). Nonetheless, given that many shoot-feeding insects have evolved the capacity to cope with plant defensive compounds and in some cases exploit them for host plant location (Bernays and Chapman, 1994), it seems likely that this occurs with soil-dwelling insects too. Moreover, Robert et al. (2012) also noted that root-feeding was positively correlated with concentrations of phenolic acids (e.g., chlorogenic acid), which also has been seen for the vine weevil (*O. sulcatus*) that feeds on the roots of raspberry (Clark et al., 2011) and blackcurrant (Johnson et al., 2011).

In addition to root exudates (usually volatiles) used by insect herbivores to locate and distinguish host plants from a distance, there is a whole range of contact chemosensory compounds that can either stimulate or deter feeding once initial herbivory has started (see Table 3 in Johnson and Gregory, 2006). To our knowledge, there have been no significant changes to this list, and the behavior of soil-dwelling herbivores seems generally similar to insect herbivores feeding aboveground (Chapman, 2003). For example, most of the ‘phagostimulants’ reported

Table 2 Chemical cues exuded by roots in the rhizosphere that enable root-feeding insects to locate host-plants, adapted from Johnson and Gregory (2006). Insect Orders are (D) Diptera and (C) Coleoptera. 'Type' describes the nature of the chemical cue; (*es*) esters, (*ke*) ketones, (*ad*) aldehydes, (*ie*) isothiocyanate, (*ac*) alcohols, (*ca*) carboxylic acids, (*aa*) amino acids, (*mc*) mercaptans, (*hy*) hydrocarbons, (*is*) isoflavonoids and (*o*) others. Effect refers to whether the chemical is an 'attractant' (+) or a 'repellent' (−)

Insect order	Insect species	Chemical compound	Type	Effect	Reference
D	Carrot root fly *Psilae rosae*	methyl eugenol	*o*	+	(Jones and Coaker, 1977, 1979)
		bornyl acetate	*es*	+	(Ryan and Guerin, 1982; Guerin and Ryan, 1984)
		2,4-dimethyl styrene	*hy*	+	
		α-ionone	*ke*	+	
		β-ionone	*ke*	+	
		biphenyl	*hy*	+	
		falcarinol	*o*	+	(Maki et al., 1989; Maki and Ryan, 1989)
		falcarindiol	*o*	+	
		falcarindiol monoacetate	*o*	+	
		trans-2-nonenal	*ad*	−	(Guerin and Ryan, 1984)
D	Cabbage root fly *Delia radicum*	isothiocyanates[a]	*ie*	+/−	(Finch and Skinner, 1974)
		allyl isothiocyanate	*ie*	+	(Koštál, 1992; Ross and Anderson, 1992)
		ethyl isothiocyanate	*ie*	+	
		n-dipropyl disulphide	*o*	+	(Ross and Anderson, 1992)
		allyl alcohol	*o*	+	
		methyl eugenol	*o*	+	
		hexanol	*ac*	+	(Koštál, 1992)
		hexanal	*ad*	+	
		cis-3-hexen-1-ol	*ac*	+	
		linalool	*ac*	+	
		hexylacetate	*o*	−	
		cis-3-hexenyl acetate	*o*	−	
		benzaldehyde	*o*	−	
		myrcene	*hy*	−	
		terpinene	*hy*	−	
		α-pinene	*hy*	−	
		limonene	*hy*	−	
D	Onion root fly *Delia antiqua*	*n*-propyl disulphide	*o*	+	(Matsumoto and Thorsteinson, 1968; Ross and Anderson, 1992)
		methyl disulphide	*o*	+	
		n-propyl mercaptan	*mc*	+	
		ethyl acetate	*es*	+	(Ikeshoji et al., 1980)
		tetramethylpyrazine	*o*	+	
		n-heptanal	*ad*	+	
		propanol	*ac*	+	(Mochizuki et al., 1989)
		butanol	*ac*	+	
		pentanol	*ac*	+	
		hexanol	*ac*	+	
		heptanol	*ac*	+	
		pentanal	*ad*	+	
		hexanal	*ad*	+	
		heptanal	*ad*	+	
		valeric acid	*ca*	+	
		caproic acid	*ca*	+	
		enanthic acid	*ca*	+	
		21 esters[b]	*es*	+	
		allyl-isothiocyanate	*ie*	+	(Ross and Anderson, 1992)
		2-phenyl ethanol	*ac*	−	
		n-dipropyl disulphide	*o*	+	
		sulphur compounds[c]	*o*	+/−	(Soni and Finch, 1979)

Table 2 (continued)

Insect order	Insect species	Chemical compound	Type	Effect	Reference
		ethyl sulphide	*o*	+	(Matsumoto, 1970)
		n-butyl sulphide	*o*	+	
		iso-butyl sulphide	*o*	+	
		n-butyl methyl sulphide	*o*	+	
		n-butyl ethyl sulphide	*o*	+	
		iso-pentyl sulphide	*o*	+	
		allyl sulphide	*o*	+	
		n-propyl sulphide	*o*	+	
D	Turnip root fly	allyl-isothiocyanate	*ie*	+	(Rygg and Sömme, 1972; Ross and Anderson, 1992)
	Delia floralis	methyl eugenol	*o*	+	
		n-dipropyl disulphide	*o*	+	(Ross and Anderson, 1992)
		allyl alcohol	*o*	+	
		phenylethyl-isothiocyanate	*ie*	−	(Rygg and Sömme, 1972)
C	Clover root borer	estragole	*o*	+	(Kamm and Buttery, 1984)
	Hylastinus obscurus	pentadecanal	*ad*	+	
		hexadecanal	*ad*	+	
		hexanoic acid	*ca*	+	
		ethyl laurate	*es*	+	
		ethyl benzoate	*es*	+	
		E-2-hexenal	*ad*	+	(Tapia et al., 2007)
		methyl benzoate	*es*	+	
		limonene	*hy*	−	
C	Pine weevil	α-pinene	*hy*	+	(Nordenhem and Nordlander, 1994)
	Hylobius abietis	ethanol	*ac*	+	
C	Wireworms	ethyl acetate	*es*	+	(Morgan and Crumb, 1928)
	Agriotes spp.	nitrobenzene	*o*	+	
		aspartic acid	*aa*	+	(Thorpe et al., 1946)
		asparagine	*aa*	+	
		malic acid	*ca*	+	
		succinic acid	*ca*	+	
		glutamine	*aa*	+	
		glutamic acid	*aa*	+	
C	Bark beetle	α-pinene	*hy*	+	(Rudinsky, 1966; Rudinsky and Zethner-Møller, 1967)
	Hylastus nigrinus	β-pinene	*hy*	+	
		camphene	*hy*	+	
C	Cockchafer *Melolontha hippocastani*	Monoterpenes[d]	*hy*	+	(Weissteiner and Schutz, 2006)
C	Clover root weevil *Sitona lepidus*	Fomononetin	*is*	+	(Johnson et al., 2005)
C	Western corn rootworm *Diabrotica virgifera virgifera*	1,4-benzoxazin-3-1 derivatives, including 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA)		+	(Robert et al., 2012)

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[a] Finch and Skinner (1974) report several unspecified isothiocyanates that are either attractive or repellent to the cabbage root fly, *Delia radicum*

[b] Mochizuki et al. (1989) list 21 closely related esters (not shown) that are attractive to the onion root fly, *Delia antiqua*. Esters with seven carbon atoms or less were attractive, those with eight or more were not

[c] Soni and Finch (1979) report 15 sulphur compounds that are either attractive or repellent to the onion root fly, *Delia antiqua*, depending on concentration. In addition to those described in Matsumoto and Thorsteinson (1968) that are present in onions (*Allium cepa*), Soni and Finch (1979) list sulphur compounds that are attractive to *D. antiqua* but are not known to be present in *A. cepa*

[d] Weissteiner and Schutz (2006) reported selective preference for carrot roots which primarily released monoterpenes, whereas less attractive oak roots emitted fatty acids. It was not established which compounds in the blend were attractive

for root herbivores are sugars (50 %), whereas secondary compounds tend to 'deter' further feeding. The isoflavonoids, in particular, have been reported as having deterrent effects on generalist root herbivores (Sutherland et al., 1980; Russell et al., 1982; Lane et al., 1985; Gaynor et al., 1986). However, as discussed above, one isoflavonoid (formononetin) had 'attractive' properties for the specialist clover root weevil (*Sitona lepidus*) (Johnson et al., 2005). Given the strong affinity of this species with the root nodules of white clover (*Trifolium repens*) tissues (Gerard, 2001), which contain significant levels of formononetin (Mathesius, 2001), it seems likely that the clover root weevil may have overcome any deterrent effects. Moreover, formononetin may be relatively benign (and, therefore, easier to adapt to) given that Sutherland et al. (1980) reported that neither the grass grub (*Costelytra zealandica*) nor the African black beetle (*Heteronychus arator* F.) were negatively affected by this compound.

Comparing Chemical Cues Used by Insect and Nematode Herbivores

The chemical ecology of nematodes is reviewed by Rasmann et al. (2012, this issue), but here we consider whether insects and nematodes show any similarities in terms of chemically mediated orientation to roots. Like insect herbivores, plant parasitic nematodes have been found to use CO_2 as a 'long-distance' kairomone for root location (Klingler, 1961; Prot, 1980). Theoretically this can act up to 1 m away for a single root and >2 m for a root mass (Dusenbery, 1987). Apart from CO_2, our ability to compare insect and nematode responses to plant metabolites is limited to a few specific compounds. Specifically, DIMBOA and glutamic acid tend to 'attract' both nematodes (Riga et al., 1997; Friebe et al., 1998) and insects (see Table 2). Similarly, ascorbic acid 'attracts' nematodes (Bird, 1959, 1962) and acts as a 'phagostimulant' to the grass grub *C. zealandica* (Sutherland and Hillier, 1974a). By contrast, cucurbitacin A, a bitter triterpenoid compound from cucumber, 'repels' *Meloidogyne incognita* (Haynes and Jones, 1976; Chitwood, 2002) while it acts as a 'phagostimulant' to the curcurbit beetle (Eben et al., 1997). The response to these compounds is, however, rather idiosyncratic as highlighted by the study by Riga et al. (1997), which showed that male *Globodera rostochiensis* and *G. pallida* were 'attracted' to *L*-glutamic acid but not *D*-glutamic acid.

Is Insect Orientation Affected by Nematode Herbivory?

The role of micro-organisms in allelopathy is reviewed by Cipollini et al. (2012, this issue). However, considering the overlap in resources utilized by insect and nematode herbivores, the question arises: 'Do plant parasitic nematodes influence the behavior of insect herbivores and *vice versa*?' In particular, will feeding on roots by one group enhance or reduce the attractiveness of a host to the other group?

Despite the potentially destructive impact that arises from simultaneous attack by plant parasitic nematodes and insect herbivores (Blackshaw and Kerry, 2008), very few studies have investigated whether these two groups interact through chemical cues from plants. One of the few illustrative examples of an interaction between herbivore induced plant chemical responses and the two groups of herbivores is outlined in the paper by Ali et al. (2011). They showed how *Citrus aurantium* and *Citrus paradisi* × *Ponsirus trifoliata*, induce the production of Pregeijerene and Geijerene (a terpene and associated breakdown product) in response to herbivory by the root weevil *Diaprepes abbreviatus*. These compounds are known to 'attract' entomopathogenic nematodes, antagonists of the root weevil, but the authors found that they also 'attract' the plant parasitic nematode *Tylenchulus semipenetrans*, which is a major pest on citrus plants (Ali et al., 2011). In short, while the release of herbivore induced plant volatiles may help ameliorate the attack of one type of herbivore by signaling for its pathogens, it may induce the attack of another type of herbivore.

There is further evidence that suggests that the two groups of herbivores can potentially interact through plant responses to herbivory. In particular, juveniles of some nematode species, such as *G. rostochiensis*, show complete dependence on the presence of plant root exudates for hatching (Perry, 1997), and *Heterodera schachtii* second stage juveniles show oriented searching in the presence of root exudates (Clemens et al., 1994; Perry and Aumann, 1998). Hence, higher concentrations of exudates in the soil caused by insect herbivores may trigger the hatching of nematodes and increase the pathogen load on the plant. Similarly, plant parasitic nematodes may influence the release of plant derived organic compounds into the soil solution, and through this influence the orientation of insect herbivores. It has, for example, been shown that feeding on white clover roots by *H. trifolii* and other nematodes increases the amount of photosynthetically derived C in the microbial biomass (Yeates et al., 1999), and feeding by *M. incognita* increases concentrations of non-volatile water soluble ^{14}C and several metal ions (Ca, Mg, Na, K, Fe, Cu) in root exudates from tomato plants (Van Gundy et al., 1977). This indicates that plant parasitic nematodes can increase the concentration of plant derived C in the soil solution, and it seems likely that insect herbivores would respond to the increased concentrations of root exudates caused by nematode herbivory. Whether an insect herbivore will be attracted or repelled through this will, however, depend on what other plant metabolites are being released. For instance, a study that investigated the influence of nematodes (not limited to plant parasitic nematodes) on *Plantago lanceolata* found that when nematodes were present in the soil *P. lanceolata* increased the concentration of aucubin and catalpol (iridoid glucosides) in the root exudates (Wurst et al., 2010). These two compounds are known to be broadly toxic to, or at least deter, generalist herbivores aboveground (Dobler et al., 2011), and it seems likely that a similar effect would be found for insect herbivores belowground. However, some specialist herbivores aboveground are known to sequester iridoid glucosides, thus reducing their palatability to predators (Dobler et al., 2011), and we cannot rule out a similar effect in belowground specialist insect herbivores.

The Rhizosphere and Chemical Signals

As discussed elsewhere in this issue (Effmert et al., 2012; Hartmann and Schikora, 2012; Hiltpold and Turlings, 2012; Jung et al., 2012) the rhizosphere represents a very different medium for chemical signaling than aerial parts of the plant. The condition of the soil in terms of porosity, moisture content, and bulk density will affect both the diffusion of chemicals and the behavior of insects. As Johnson and Gregory (2006) point out, a gaseous molecule can diffuse through 1 m of air more rapidly than through 1 mm film of water within a soil pore (Payne and Gregory, 1988), and increasing soil bulk density from 1.1 Mg m^{-3} to 1.5 Mg m^{-3}, reduces mobility of western corn root worms by 90 % (Strnad and Bergman, 1987b). Based on mathematical models of belowground insect herbivore orientation to host plants (Zhang et al., 2006) and later (Zhang et al., 2007), Johnson and Gregory (2006) suggested a conceptual model for host plant location by such herbivores. Essentially, in the absence of relevant semiochemicals, insects move in a random manner (Zhang et al., 2006), but in the presence of generic signals (e.g., CO_2) begin to search localized patches more intensively (e.g., Johnson et al., 2006). In the case of specialist feeders, attraction and orientated movement becomes evident with more specific chemical signals followed by feeding stimulation or deterrence at the root interface, determined by contact chemosensory signals.

Future Challenges and Conclusions

The recent observation that root-herbivores might be positively affected by some secondary compounds, and phenolics in particular (Clark et al., 2011; Johnson et al., 2011; Robert et al., 2012), highlights how we still know relatively little about the role of root defenses against root herbivory (comprehensively discussed by van Dam, 2009). Given that there are numerous examples of shoot herbivores adapting to, and even exploiting defensive chemicals for host plant selection (Bernays and Chapman, 1994), it seems intuitive that root herbivores should do the same. While phenolic compounds seldom have positive effects on shoot herbivores (for exceptions, see Bernays and Woodhead, 1982; Bernays et al., 1983), it remains possible that feeding on plant tissues with low nitrogen concentrations (usually the case in roots) causes herbivores to adaptively exploit phenolics for physiological development. For example, the grasshopper, *Anacridium melanorhodon*, conserves available nitrogen by using phenolics for cuticle sclerotization (Bernays and Woodhead, 1982). In attempting to better characterize how root herbivores respond to root chemistry, it may become clearer whether compounds regarded as having defensive roles against shoot herbivores have the same function belowground.

There also is a gap in our knowledge about the interactive effects between insect and nematode herbivores belowground, and a clear need for further investigation into their potential interactions. To promote this we need more studies that quantify the release of specific herbivore induced plant compounds in response to specific organisms, and whether the release of such compounds increase or decrease the attractiveness of a plant host to another type of herbivore. Such knowledge will perhaps enhance our capability to manage populations of soil-dwelling herbivore pests and thus secure optimal output.

In terms of the future challenge of global climate change, elevated air temperature and atmospheric CO_2 concentrations are unlikely to have direct effects on signaling and root-feeding insects (Staley and Johnson, 2008). Predicted increases in the concentration of atmospheric CO_2 will still be well below current concentrations in the soil (Payne and Gregory, 1988), so it seems unlikely that this will interfere with CO_2 attractants. Likewise, the buffering effects of soil will minimize the effects of elevated air temperatures (Staley and Johnson, 2008), although it may exacerbate the effects of lower precipitation patterns which would increase soil porosity. Greater porosity significantly increases diffusion rates of gaseous root exudates, but impairs diffusion of chemicals in solution (Payne and Gregory, 1988), which may, therefore, alter chemical signaling between roots and herbivores. Indirect (i.e., plant-mediated) effects of climate change on root signaling with belowground herbivores may be envisaged. For example, it generally is thought that elevated CO_2 promotes root biomass relative to shoot biomass (Rogers et al., 1994, 1996), so it might reasonably be expected that some phagostimulants and/or deterrents may become diluted in root tissues (but see Staley and Johnson, 2008 for exceptions) resulting in altered rates of root herbivory. In terms of the effects of elevated CO_2 on semio-chemicals in the rhizosphere, this is likely to be highly system specific. For example, elevated atmospheric CO_2 concentrations increased production of rhizobial root nodules in white clover (*T. repens*) with corresponding increases in clover root weevil (*S. lepidus*) populations and development rates (Johnson and McNicol, 2010). Given the attraction of *S. lepidus* to rhizobial root nodules (Gerard, 2001), it seems likely that greater numbers of nodules would increase overall concentrations of the chemical cues underpinning this attraction.

This review has shown the breadth of chemicals that elicit behavioral responses in root-feeding insects. To date, little attempt has been made to exploit this research to manage pest populations despite some tentative evidence of success (e.g., Bernklau et al., 2004). 'Enemy recruitment chemicals' that some insects elicit in their host plants (Rasmann et al., 2012, this issue), show potential application in pest management (Hiltpold and Turlings, 2012, this issue). We suggest that host plant location cues might also play a role in the management of subterranean insect pests, and represent an, as yet, untapped area of chemical ecology.

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Ecology and Evolution of Soil Nematode Chemotaxis

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Abstract Plants influence the behavior of and modify community composition of soil-dwelling organisms through the exudation of organic molecules. Given the chemical complexity of the soil matrix, soil-dwelling organisms have evolved the ability to detect and respond to these cues for successful foraging. A key question is how specific these responses are and how they may evolve. Here, we review and discuss the ecology and evolution of chemotaxis of soil nematodes. Soil nematodes are a group of diverse functional and taxonomic types, which may reveal a variety of responses. We predicted that nematodes of different feeding guilds use host-specific cues for chemotaxis. However, the examination of a comprehensive nematode phylogeny revealed that distantly related nematodes, and nematodes from different feeding guilds, can exploit the same signals for positive orientation. Carbon dioxide (CO_2), which is ubiquitous in soil and indicates biological activity, is widely used as such a cue. The use of the same signals by a variety of species and species groups suggests that parts of the chemo-sensory machinery have remained highly conserved during the radiation of nematodes. However, besides CO_2, many other chemical compounds, belonging to different chemical classes, have been shown to induce chemotaxis in nematodes. Plants surrounded by a complex nematode community, including beneficial entomopathogenic nematodes, plant-parasitic nematodes, as well as microbial feeders, are thus under diffuse selection for producing specific molecules in the rhizosphere that maximize their fitness. However, it is largely unknown how selection may operate and how belowground signaling may evolve. Given the paucity of data for certain groups of nematodes, future work is needed to better understand the evolutionary mechanisms of communication between plant roots and soil biota.

Keywords Carbon dioxide · *Caenorhabditis elegans* · Chemotaxis · Entomopathogenic nematodes · Functional phylogenetic · Nematode · Plant-parasitic nematodes · Sense organs · Soil chemical ecology

Introduction

All animals depend on green plants, either directly or indirectly, as their primary source of energy. Primary consumers need plants for nourishment, whereas secondary consumers, such as predators or parasites may use plant cues to locate their herbivore hosts. Several decades of work have shown that animals use plant-derived physical (visual, tactile), and/or chemical (olfactory, gustatory) cues to locate and accept their food source (Schoonhoven et al., 2005). Most of these multitrophic interactions studies have been done using

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aboveground communities (Price et al., 1980; Vet and Dicke, 1992; Tscharntke and Hawkins, 2002). However, the last decade has shown a marked increase in exploring interactions between plants and soil animals (e.g., Strong et al., 1999; Gange and Brown, 2002; De Deyn et al., 2003; Van der Putten, 2003; de la Peña et al., 2006; Rasmann and Agrawal, 2008; Bonkowski et al., 2009), and how plants mediate interactions between aboveground and belowground communities (Van der Putten et al., 2001; Wardle, 2002; Bezemer and van Dam, 2005; Erb et al., 2008; Kaplan et al., 2008; van Dam, 2009). Indeed, roots often are the storage site for nutrients and metabolites, are a place where energy from photosynthesis can be stored, and can act as a shelter for soil-dwelling organisms (Hunter, 2001). Therefore, roots are a hub for ecological interactions that ultimately influence almost all groups of soil inhabitants (Coleman, 1976; Fogel, 1985; Walker et al., 2003; Whittaker, 2003).

General patterns and theories for chemically-mediated interactions that structure aboveground communities (Price et al., 1980; Vet and Dicke, 1992; Karban and Baldwin, 1997) also can be applied belowground (Strong et al., 1999; van Tol et al., 2001; van der Putten et al., 2009; Rasmann et al., 2011a). In fact, as aboveground, plants can influence the behavior, abundance, and composition of soil animal communities (e.g., Yeates, 1999; Buyer et al., 2002; Viketoft et al., 2009), and can mediate indirect interactions between organisms of different trophic levels (Coleman, 1976; Strong et al., 1999). However, striking differences exist between above- and belowground subsystems (see also Erb et al., 2012, this issue).

The soil matrix is composed of all three phases (gas, liquid, and solid), which can impact mobility, behavior, signaling, and interaction between organisms in a different manner than when living aboveground. This will influence the mobility of organisms belowground, often not surpassing more than 1 m^2 in their lifetime, whereas aboveground, vertebrates can explore more than 1 ha per day (Hedlund et al., 2004). Moreover, the physico-chemical legacy of the soil, shaped by high metabolic retention capacity, slow turnover of chemical metabolites, slower diffusion, and higher heterogeneity suggests a slower rate for ecological interactions to occur (Coleman et al., 2004). Roots themselves also have a very different physiology, which may lead to distinct patterns of interactions belowground compared to aboveground (see Erb et al., 2012, this issue). All this together should impose selection for particular stimuli to be perceived by soil organisms. For example, soil inhabitants are strongly limited in their use of visual information, but rather use chemical and tactile cues to communicate and behave (Jones, 2002). Roots can produce and exude into the rhizosphere a great variety of compounds ranging from amino acids, complex polysaccharides, and proteins, to smaller, more volatile lipophilic molecules, all of which also have been shown to directly or indirectly influence the soil community of organisms (Bais et al., 2006). The aim of our review is to list and discuss published information on how plant chemical exudates can influence the ecology and evolution of host-searching and foraging strategies of soil-borne nematodes (but see also Johnson and Nielsen (2012), this issue for discussion on belowground herbivorous insects).

We particularly focus on soil nematodes because they are among the most diverse groups of soil organisms. Each square meter of soil may contain millions of individual nematodes belonging to over 400 species (Gaugler and Bilgrami, 2004). There are at least seven functional types, including: plant feeding nematodes (e.g., the genera *Pratylenchus, Heterodera, Meloidogyne, Helicotylenchus, Aphelenchoides*), plant-associated nematodes (e.g., the genera *Tylenchus, Dorylaimellus*), fungal hyphae-feeding nematodes (e.g., the genera *Aphelenchus, Aphelenchoides, Leptonchus, Diphtherophora*), bacterial feeding nematodes (e.g., the genera *Rhabditis*, *Plectus, Cephalobus*, *Caenorhabditis*), nematodes that feed on unicellular eukaryotes, animal-parasitic nematodes (e.g., the genera *Heterorhabditis*, *Steinernema*), and omnivorous nematodes (e.g., the order *Dorylaimida*) (Yeates, 1999). These functional types are taxonomically heterogeneous, and most likely the result of convergent evolution (e.g., Blaxter et al., 1998; Holterman et al., 2006). Additionally, it is worth noting that more than one feeding habit can occur within a genus (e.g., the genus *Aphelenchoides* harbors fungivorous and plant-parasitic species), or even within a single individual (Yeates, 1999).

Because of their abundance, systematic and functional diversity, and their representation in multiple trophic levels in the soil food web, nematodes have strong influences on ecosystem dynamics and functioning (Yeates et al., 2009). They have been shown to influence soil nutrient cycling, growth rate, health, and yield of plants as well as populations of other soil inhabitants. For example, herbivores influence plant yield (Chitwood, 2002), plant community composition (De Deyn et al., 2004), and successional dynamics (Mortimer et al., 1999; De Deyn et al., 2003). Microbial feeders can stimulate nutrient cycling and plant yield (Ingham et al., 1985; Fu et al., 2005). Detritivorous nematodes can contribute up to 40 % of total organic matter mineralization (De Ruiter et al., 1993), and predators and parasites can reduce arthropod populations (e.g., Kaya and Gaugler, 1993), which in turn can have cascading effects on plant performance (Strong et al., 1999; Rasmann et al., 2011b).

Below, we review literature of nematode sensory machinery and chemically-mediated orientation toward organic molecules. Little is known of how chemotaxis has evolved during the radiation of nematodes. Chemotaxis is the directed orientation of the nematode toward or away from the source of stimulation (in our case the plants). Using a comprehensive phylogeny of nematodes, we map chemical compounds that have been proven to produce taxis. This will lead to preliminary conclusions on how chemotaxis can evolve in soil nematodes. Finally, we discuss how plants can structure communities of nematodes through root exudation and how this may operate to their own benefit.

The Sensory Apparatus of Soil Nematodes

As in all animals, nematode behavior is the coordinate integration of several external stimuli leading to responses (e.g., locomotion, movement, feeding, mating, penetration) (Gaugler and Bilgrami, 2004). Nematodes' sensory apparatus allows them to use chemical, electrical, light, mechanical, and temperature stimuli (Jones, 2002) to orientate, move, and locate a sexual partner, as well as energy sources (food) in the soil (Lee, 2002).

Nematode sense organs basically can be subdivided into cuticular and internal sense organs. Cuticular sense organs generally are composed of a sheath cell, a socket cell, and a variable number of dendritic processes, and are responsible for detecting chemical, mechanical and temperature related stimuli. Internal sense organs are more diverse, mainly responsible for detecting mechanical stimuli as well as light stimuli (Jones, 2002). Invariably, the largest and most complex of the nematode sense organs are the amphids, which are exposed to the external environment by a pore in the cuticule, primarily functioning as chemoreceptors. In *Caenorhabditis elegans*, the tail bilateral sensory organs called phasmids also are shown to function as chemoreceptors and help the nematode orientate towards or away from the chemical stimuli (Hilliard et al., 2002). Numerous nematode taxa do not have phasmids (e.g., members of Clade 1–6 according to Holterman et al. (2006)). Because of the complexity and abundance of soil chemicals compared to other physical stimuli, chemoreception is undoubtedly the most important source of stimulus to nematodes (Jones, 2002).

Responses by nematodes to chemical stimuli have been extensively studied in the bacteriophagous nematode *C. elegans*, some plant-parasitic (e.g., *Meloidogyne* and *Globodera* spp.), and in an increasing number of animal-parasitic nematodes (e.g., *Heterorhabditis* and *Steinernema* spp.). Among the genera mentioned above, *C. elegans* has an uncommon ecology: it is present only in nutritionally very rich habitats (e.g., mature compost heaps), and is seldom found in 'normal' soils. Hence, some restraint in the extrapolation of *C. elegans* data to other genera would be justified.

Chemotaxis in Nematodes

In sections below and in Table 1, we summarize major chemo-attractants for soil nematodes. The high occurrences of a wide variety of compounds known to mediate changes in nematode behavior for particular species (e.g., *C. elegans*) are likely due to research bias towards model species. For example, because of the ability to map gene-level responses with behavior, studies of attraction/repulsion to/from allelochemicals in *C. elegans* comprise most of the work done on all nematodes (Bargmann and Mori, 1997). Although some compounds have been identified as potent nematode repellents, such as, D-tryptophan, α-terthienyl, high levels of CO_2, copper and zinc ions, and inositol (Balanova and Balan, 1991; Ward, 1978), we focused on plant produced kairomones, which stimulate positive orientation, and also because most studies assess positive orientation in nematode bioassays. This will set the stage for discussing evolutionary ecology of plant-nematode interactions.

Chemotaxis of Plant-Parasitic Nematodes Plant-parasitic nematodes can be divided into broad groups based on the plant parts they infest. Foliar nematodes (*Aphelenchoides* sp.) move into shoots and invade leaf buds causing necrosis and deformation of plant leaves. The stem nematodes (*Ditylenchus dipsaci)* cause malformations, decline in growth, and dry rot in above- and belowground parts of stems. Other nematodes infect roots and cause growth reduction in whole plants and malformations in underground plant parts (*Meloidogyne* spp., *Rotylenchus uniformis)*, root necrosis, and growth reduction (*Pratylenchus penetrans, Tylenchulus semipenetrans*), or growth reduction without any obvious or typical symptoms (*Globodera rostochiensis, G. pallida*, and *Tylenchorhynchus dubius)*. Here, we limit our analysis of allelochemicals that affect soil-dwelling plant-parasitic nematodes, which mainly exploit plant roots as their only source of nutrients. Species of plant-parasitic nematodes may spend their whole life cycle outside the plant, feeding from the surface or deeper tissues, while others have the capacity to invade the root and feed from cortical cells. In many cases, feeding cells are transformed into highly specialized feeding structures to support nematode development and reproduction such as for cyst (e.g., *Heterodera* and *Globodera* spp.) and root-knot nematodes (*Meloidogyne* spp.) (Wyss, 2002). Both these so-called sedentary endoparasites are economically important because of their ability to cause damage to major crop species.

Different control mechanisms underlie the hatching of cyst and root knot nematodes. Root knot nematodes in general have a far broader host range than cyst nematodes. Because of their specificity, it is essential for cyst nematodes to hatch in the direct vicinity of a suitable host plant, instead of near any plant species. Cyst nematode hatching is triggered by a complex mixture of components released by the roots of host plants in a species-dependent manner (Prot, 1980). There is a variable degree of dependence of cyst

Table 1 Attractive chemical compounds for different trophic guilds of soil nematodes. Shown are nematodes species grouped in three different guilds (bacteriophagous, entomopathogenic, and plant-parasitic) and their corresponding chemical attractant. Choice of the references is based on whether the study correlated actual nematode behavior with individual chemical compounds present. We excluded all compounds that stimulated repulsion, but we acknowledge that different concentrations of the same compounds can be either attractive or repulsive (see text). Note that entomopathogenic nematodes are functionally bacteriophagous, but cannot grow and reproduce outside the arthropod protective shell, making them unique in regard of their guild

Feeding guilds and nematode species	Attractive compounds	Compounds' type	References
Bacterivorous			
Caenorhabditis elegans	2,3 butanedione	ketone	(Hallem et al., 2011)
	2-butanone		(Hallem et al., 2011)
	2-pentanone		(Hallem et al., 2011)
	3-carene	terpenes	(Hallem et al., 2011)
	4,5 dimethylthiazole	thiazoles	(Hallem et al., 2011)
	α-humulene	terpenes	(Hallem et al., 2011)
	α-pinene		(Hallem et al., 2011)
	benzothiazole	thiazoles	(Hallem et al., 2011)
	cAMP	cAMP	(Bird, 1960)
	carbon dioxide	atmospheric gaz	(Bird, 1960)
	ethylacetate	acids	(Hallem et al., 2011)
	ions	ions	(Ward, 1978)
	linalool	terpenes	(Hallem et al., 2011)
	methyl acetate	acids	(Hallem et al., 2011)
	octadecanoid acid		(Hallem et al., 2011)
	propanol	alchools	(Hallem et al., 2011)
	trimethylamine	amines	(Hallem et al., 2011)
Entomopathogen			
Heterorhabditis bacteriophora	(*E*)-β-caryophyllene	terpenes	(Rasmann et al., 2005)
	1-heptanol	alcohols	(O'Halloran and Burnell, 2003)
	1-hexanol		(O'Halloran and Burnell, 2003)
	1-nonanol		(O'Halloran and Burnell, 2003)
	1-octanol		(O'Halloran and Burnell, 2003)
	1-pentanol		(O'Halloran and Burnell, 2003)
	2-acetylthiazole	thiazoles	(O'Halloran and Burnell, 2003)
	2-heptanol	alcohols	(O'Halloran and Burnell, 2003)
	2-isobutylthiazole	thiazoles	(O'Halloran and Burnell, 2003)
	2-methylpyrazine	pyrazines	(O'Halloran and Burnell, 2003)
	2-nonanol	alcohols	(O'Halloran and Burnell, 2003)
	2-octanol		(O'Halloran and Burnell, 2003)
	3-nonanol		(O'Halloran and Burnell, 2003)
	4,5 dimethylthiazole	thiazoles	(Hallem et al., 2011)
	4,5-dimethylthiazole	thiazoles	(O'Halloran and Burnell, 2003)
	benzothiazole	thiazoles	(O'Halloran and Burnell, 2003)
	caproic acid	acids	(O'Halloran and Burnell, 2003)
	caprylic acid		(O'Halloran and Burnell, 2003)
	carbon dioxide	atmospheric gaz	(O'Halloran and Burnell, 2003)
	methy salicilate	aromatic compound	(Hallem et al., 2011)
	methylvaleric acid	acids	(O'Halloran and Burnell, 2003)
	p-cymene	terpenes	(Hallem et al., 2011)
	propanol	alcohols	(Hallem et al., 2011)
	undecyl acetate	acids	(Hallem et al., 2011)
H. indica	geijerene	terpenes	(Ali et al., 2011)
	pregeijerene		(Ali et al., 2011)

Table 1 (continued)

Feeding guilds and nematode species	Attractive compounds	Compounds' type	References
H. megidis	(*E*)-β-farnesene		(Kollner et al., 2008)
	(*E*)-nerolidol		(Kollner et al., 2008)
Steinernema carpocapsae	2-nonanone	ketone	(Hallem et al., 2011)
	4,5 dimethylthiazole	thiazoles	(Hallem et al., 2011)
	carbon dioxide	gaz	Gaugler et al. 1980
	heptanol	alcohol	(Hallem et al., 2011)
	hexanol		(Hallem et al., 2011)
	nonanol		(Hallem et al., 2011)
	octanol		(Hallem et al., 2011)
	octyl acetate	acids	(Hallem et al., 2011)
	pentanol	alcohols	(Hallem et al., 2011)
S. diaprepsi	α-santalene	terpenes	(Ali et al., 2011)
S. feltiae	α-santalene		(Ali et al., 2011)
S. glaseri	carbon dioxide	gaz	(Robinson, 1995)
S. riobrave	α-santalene	terpenes	(Ali et al., 2011)
Plant-parasite			
Aphelenchoides fragariae	carbon dioxide	gaz	(Bird, 1960)
A. ritzemabosi	carbon dioxide		(Klinger, 1970)
Ditylenchus dipsaci	carbon dioxide		(Pline and Dusenbery, 1987)
Globodera pallida	g-aminobutyric acid	acids	(Riga, 2004)
	L-glutamic acid		(Riga, 2004)
G. rostochiensis	a-aminobutiric acid		(Riga, 2004)
	L-glutamic acid		(Riga, 2004)
Heterodera schactii	carbon dioxide	gaz	(Bird, 1960)
Meloidogyne incognita	carbon dioxide		(McCallum and Dusenbery, 1992)
M. javanica	carbon dioxide		(Pline and Dusenbery, 1987)
Panagrellus silusiae	carbon dioxide		(Viglierchio, 1990)
Rotylenchus reniformis	cAMP	cAMP	(Riddle and Bird, 1985)
	ions	ions	(Riddle and Bird, 1985)
Tylenchulus semipenetrans	geijerene	terpenes	(Ali et al., 2011)
	ions	ions	(Abou-Setta and Duncan, 1998)
	limonene	terpenes	(Ali et al., 2011)
	pregeijerene		(Ali et al., 2011)

nematodes on these plant cues: whereas exposure to root diffusates is almost a prerequisite for the hatching of potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*), the hatching of soybean and beet cyst nematodes (*Heterodera glycines* and *H. schachtii*) is merely stimulated by such compounds. In contrast, the hatching of root knot nematodes is mainly temperature driven (Perry and Wesemael, 2008).

One of the remarkable characteristics of root diffusate-based hatching of cyst nematodes is the high activity of host plant-derived hatching stimuli ("eclepins"). The water-soluble glycinoeclepins A, B, and C (pentanor- (A) or nortriterpenes (B and C)) isolated from the roots of kidney bean are active at concentrations as low as 10^{-11}–10^{-12} g per ml (Masamune et al., 1982). The tetranortriterpene solanoeclepin A released by the roots of potato are active in the same concentration range (Schenk et al., 1999). Interestingly, glycino and solanoeclepins are chemically closely related triterpenes.

Diffusion in the liquid phase is the main mechanism responsible for spreading eclepins in soil, and eclepin-dependent cyst nematode species will hatch only in the close vicinity of a host root (cm range). It is conceivable that the freshly hatched pre-parasitic juveniles can follow relatively unspecific cues, such as CO_2 to reach the root of a host plant. Such a general signaling compound would not work for root knot nematodes, as their hatching is mainly triggered by a very general signal (*viz.* soil temperature). However, root knot nematodes are highly polyphagous. This applies especially to the most abundant species in agro-ecosystems, such as *M. incognita, M. javanica*, and *M. arenaria*. For these nematodes, following a steep (plant-derived) CO_2 gradient would imply a reasonable chance to reach a suitable plant root. The attraction of *M. incognita* by CO_2 has been shown in several studies (Dusenbery, 1987; Pline and Dusenbery, 1987). A related, alternative mechanism for host finding by root knot nematodes was proposed by Wang et al. (2009). Juveniles of *Meloidogyne hapla* had a strong preference for pH between 4.5 and 5.4, and the authors proposed root knot nematodes to be attracted by dissolved CO_2, resulting in local acidification, rather than by CO_2 itself.

For plant-parasitic nematodes other than cyst or polyphagous root knot nematodes, it is critical to exploit chemical gradients in soil that relate to the presence of a suitable host plant. For these nematodes, olfactory and other sensory organs of the nematode are essential (Huang et al., 2003). It has been acknowledged for long that plant-parasitic nematodes can locate roots of host plants in the soil (Prot, 1980) by using allelochemicals produced by the plants, as well as other soil-borne chemical compounds (Perry and Aumann, 1998). However, besides the general signal furnished by carbon dioxide (CO_2) emissions, the factors that trigger plant-parasitic nematode attraction and direction are still largely unexplored (Table 1). Carbon dioxide was shown to attract *Ditylenchus dipsaci* (Klinger, 1963; Dusenbery, 1980). Increased CO_2 levels in *Fusarium oxysporum* infested lucerne (*Medicago sativa*) roots, attracted *P. penetrans* to infected roots (Edmunds and Mai, 1967). Other, non-identified diffusates from the roots of potato increased the activity and also attracted the infective second stage juveniles of the potato cyst nematode (*G. rostochiensis*) to the roots (Perry, 1997; Devine and Jones, 2003). Similarly, *M. javanica* and G. *rostochiensis* juveniles may respond to tomato (Prot, 1980) and potato (Rolfe et al., 2000) root diffusates, respectively (reviewed in Curtis et al., 2009). The use of these attractive plant properties has been a proposed method for luring nematode pests to non-host trap crops (Franco et al., 1999). Exudates from *Asparagus officinalis* and *Tagetes erecta* are attractive to a wide range of nematodes, however, once lured in they are killed by the plants' defensive compounds (glycosides and thiophene from *A. officinalis* and *T. erecta*, respectively) (Bilgrami, 1997). Although the orientation of endoparasitic nematodes to preferred invasion sites is well established, the exact compounds in the diffusate responsible for attraction are not known (Curtis et al., 2009).

Besides CO_2, other volatile organic molecules have also been shown to serve as attractants (Table 1) or repellents for plant-parasitic nematodes, such as *M. incognita*, (McCallum and Dusenbery, 1992). Castro et al. (1989) demonstrated that volatiles from cucumber roots were attractive to *M. incognita*. Only very recently, however, it was shown that plant-parasitic nematodes can follow gradients of herbivore-induced terpenoid volatile organic compounds; *Tylenchulus semipenetrans* were more attracted to *Citrus* spp. roots infested by weevil larvae compared to uninfested plants (Ali et al., 2010, 2011). A series of terpene compounds were identified, including α-pinene, β-pinene, limonene, geijerene, and pregeijerene (Ali et al., 2011).

Chemical Ecology of Entomopathogenic Nematodes Soil-dwelling entomopathogenic nematodes comprise two families: Steinernematidae (genus *Steinernema and Neosteinernema*) and Heterorhabditidae (genus *Heterorhabditis*). They only grow and reproduce inside arthropod hosts, and third instar infective juveniles leave the cadaver. The infective juvenile (dauer juvenile) is the only stage that can survive without food for long periods while searching for alternative hosts in the soil (Gaugler, 2002). All members of both these families are actually bacteriophagous, having evolved the ability to carry and introduce symbiotic bacteria into the body cavities of insects. Bacteria then reproduce in the insect, thus furnishing the food for the nematodes to complete their life-cycle (Poinar, 1990). Because of their ability to kill the majority of insect orders and families in the soil, and the relative ease of large-scale culturing in artificial solid or liquid media, they have been promoted as exceptionally good candidates for the biological control of insect pests of roots in crop fields (Gaugler and Kaya, 1990). In general, foraging strategies of entomopathogenic nematodes can be divided into two broad categories; a cruiser form, which is highly mobile, and an ambusher form (sit-and-wait) (Campbell and Gaugler, 1997). Cruising foragers have a higher probability of finding sedentary and cryptic resources than ambushers, and ambush foragers are more effective at encountering resources with high mobility (Lewis, 2002). However, direct evidence suggests that foraging strategies used by different infective juveniles species to find a host vary along a continuum between ambush and cruise foragers (Campbell and Gaugler, 1993; Campbell and Gaugler, 1997; Lewis et al., 1992, 1993), and this behavior is plastic depending on the habitat type (Ennis et al., 2010).

Entomopathogenic nematode attraction to a suitable host can integrate different possible cues such as temperature, electric potential, carbon dioxide, and various organic and inorganic substances. However, no specific compound has been put forward for entomopathogenic nematode attraction toward the insect host (Kaya and Gaugler, 1993; Boff et al., 2001). It is generally assumed that nematode orientation and aggregation is due to unspecific signaling, such as CO_2 emissions. For example, Lewis et al. (1993) found that *S. glaseri* responded positively to volatiles cues from an insect host, and that this response was eliminated if CO_2 were removed. A similar response was later found by Grewal et al. (1994) for other cruiser *Steinernema* spp. and for two species of *Heterorhabditis*. This general response to unspecified volatile cues has been extended to many other *Steinernema* spp. (Campbell et al., 2003). On the other hand, it has been argued that CO_2 should function mainly as a short-range attractant, playing a role in host penetration through the spiracles (Ishibashi and Kondo, 1990). It also seems unlikely that such a general signal could be unambiguously exploited by foraging nematodes looking for a specific arthropod host feeding on roots. Indeed, Bilgrami et al. (2001b) found that *S. glaseri* was attracted to plant tissue from roots and leaves from *A. officinalis* and *T. erecta*, but not to nitrogenous insect products (Bilgrami et al., 2001a). Moreover, it was proposed simultaneously that entomopathogenic nematodes can use arthropod herbivore-induced plant cues to locate the site of wounding, which would automatically reveal the host (Boff et al., 2001, 2002; van Tol et al., 2001).

To date, few tritrophic interactions implying belowground herbivore-induced volatile compounds have been described, but examples include both agricultural (Rasmann et al., 2005; Ali et al., 2010, 2011) and (semi-) natural systems (Rasmann et al., 2011b). Nematodes *H. megidis*, and *H. bacteriophora* have been shown to be attracted to the sesquiterpene (*E*)-β-caryophyllene emitted by insect-damaged corn (*Zea mays*) plants (Rasmann et al., 2005; Rasmann and Turlings, 2008). Ali et al. (2010) demonstrated that citrus roots upon feeding by the root weevil *Diaprepes abbreviates* emit several terpenes including α-pinene, β-pinene, limonene, geijerene, and pregeijerene, which attracted *S. carpocapsae*, *S. diaprepesi*, *S. riobrave*, and *H. indica* from the surrounding soil. Further studies demonstrated that application of isolated HIPV pregeijerene increased larval mortality in citrus and blueberry agroecosystems by attracting naturally occuring EPN species (Ali et al. 2012 *In press*). Recently, Hallem et al. (2011) reported positive chemotaxis of *H. bacteriophora* and *S. carpocapsae* nematodes to several volatiles such as methyl salicylate, hexanol, heptanol, undecyl acetate, or 4,5-dimethylthiazole. Interestingly, they also showed that several volatiles repelled the same nematodes.

Chemotaxis and C. elegans In the bacteriophagous *C. elegans*, attraction can be mediated by a wide variety of compounds, including anions, cations, amino acids, nucleotides, variation in pH, vitamins, bacteria derived cyclic AMP, or various volatile organic compounds including the well-studied CO_2 (reviewed in Lee, 2002). Single chemosensory neurons are able to detect high and low concentrations of a single odorous compound (Sengupta et al., 1993). Also, odorant responses can adapt to various concentrations, which is reversible (Sengupta et al., 1993). Generally, responses to chemicals are dependent on developmental stage or, likely, other unknown environmental factors (Goode and Dusenbery, 1985; Riddle and Bird, 1985).

Chemotaxis and Other Nematodes Based on current systematic, ecological, and physiological knowledge, only a small fraction of nematodes are parasites of plants or animals. In fact, most nematode diversity is represented by species that are free-living in fresh water, marine, or soil systems (Baldwin et al., 2004). Free-living nematodes forage on a wide variety of substrates including bacteria, fungi, or plants. Little is known of the exact allomones that drive behavior and attraction of all other nematodes. It has been shown that secretions from fungal mycelia can attract the fungal feeder *Paurodontoides linfordi* (Klink, 1969). The free-living nematode *Panagrellus redivivus* was strongly attracted to cell-free filtrates of culture media of certain yeast and fungi, suggesting that material released by the microorganisms, such as esters or fatty acids serve as chemo-attractants (Balanova and Balan, 1991). Similarly, the free-living nematodes *Acrobeloides* sp. and *Pristionchus lheritieri* are attracted to kairomones emitted by suitable bacterial food in culture (Anderson and Coleman, 1981).

Ecology and Evolution of Soil Nematode Chemotaxis

In the complex soil matrix, in which gaseous, liquid, and solid phases can co-exists, nematodes have been shown to rely on both volatile, as well as water-soluble molecules for foraging (Bargmann and Horvitz, 1991). Indeed, it has been argued that *C. elegans* nematodes can rely on both water-soluble molecules (i.e., taste) and volatile molecules (i.e., smell) for different chemotaxis behaviors. Bargmann and Mori (1997) suggested that as volatile molecules travel quickly through diffusion and turbulence in the air, they may be used for longer-range chemotaxis, whereas water-soluble molecules are mainly used for short-range chemotaxis . For example, *H. megidis* nematodes, attracted to the corn-produced sesquiterpene (*E*)-β-caryophyllene, have been recollected at 0.5 m distance from the release point after 2 weeks (Rasmann et al., 2005, 2011b). Proportionally, to equate a nematode, humans would need to travel at 1,500 km h^{-1} to cover the same distance!

Evidence gathered in this review would suggest that both short- and long-range chemotaxis are widespread among different nematode taxa. Nematodes from different feeding guilds and from different branches of the phylogeny, indeed, utilize various, often similar, volatile, and non-volatile compounds in the soil to locate their food sources (Table 1). Undoubtedly, nematodes have evolved to sense compounds originating from a relatively long distance. However, the question is if the trait for smelling particular and possibly specific compounds may have evolved independently several times during nematode radiation. Alternatively, all nematodes may be able to smell the same molecules. In that case, the “smell” trait may be general and may have been conserved during the radiation of nematodes.

We mapped the nematode phylogeny and chemical compounds that stimulate attraction in different nematodes feeding groups in combination (Fig. 1). Such mapping showed that: 1) different feeding guilds of nematodes have repeatedly and independently evolved several times during the radiation of the group (Baldwin et al., 2004; Bert et al., 2011). This implies convergent evolution of feeding habits among soil nematodes. 2) Although we acknowledge the paucity of data, preliminary results suggest that some compounds such as CO_2 or some ions can be detected and used by a wide variety of different feeding guilds. This implies phylogenetic conservatism in chemical compound use. In other words, if traits responsible for recognition of particular compounds are conserved during the radiation of nematodes into different feeding guilds, we should then expect a broad distribution of similar compounds that can initiate a chemotaxis response, which is what we can see in Table 1, and Fig. 1.

Although respiratory emissions of CO_2 remain the most widely studied mechanism for nematode and soil-dwelling arthropod attraction (Johnson and Nielsen, 2012, this issue), this might not be the most effective mean for root location (Johnson and Gregory, 2006). In particular, in mixed stands or for specialized plant parasites, CO_2 cannot provide reliable information. Furthermore, orientation toward CO_2 gradients by the European cockchafer, *Melolontha melolontha*, disappeared when other plant-derived signals were present (Reinecke et al., 2008).

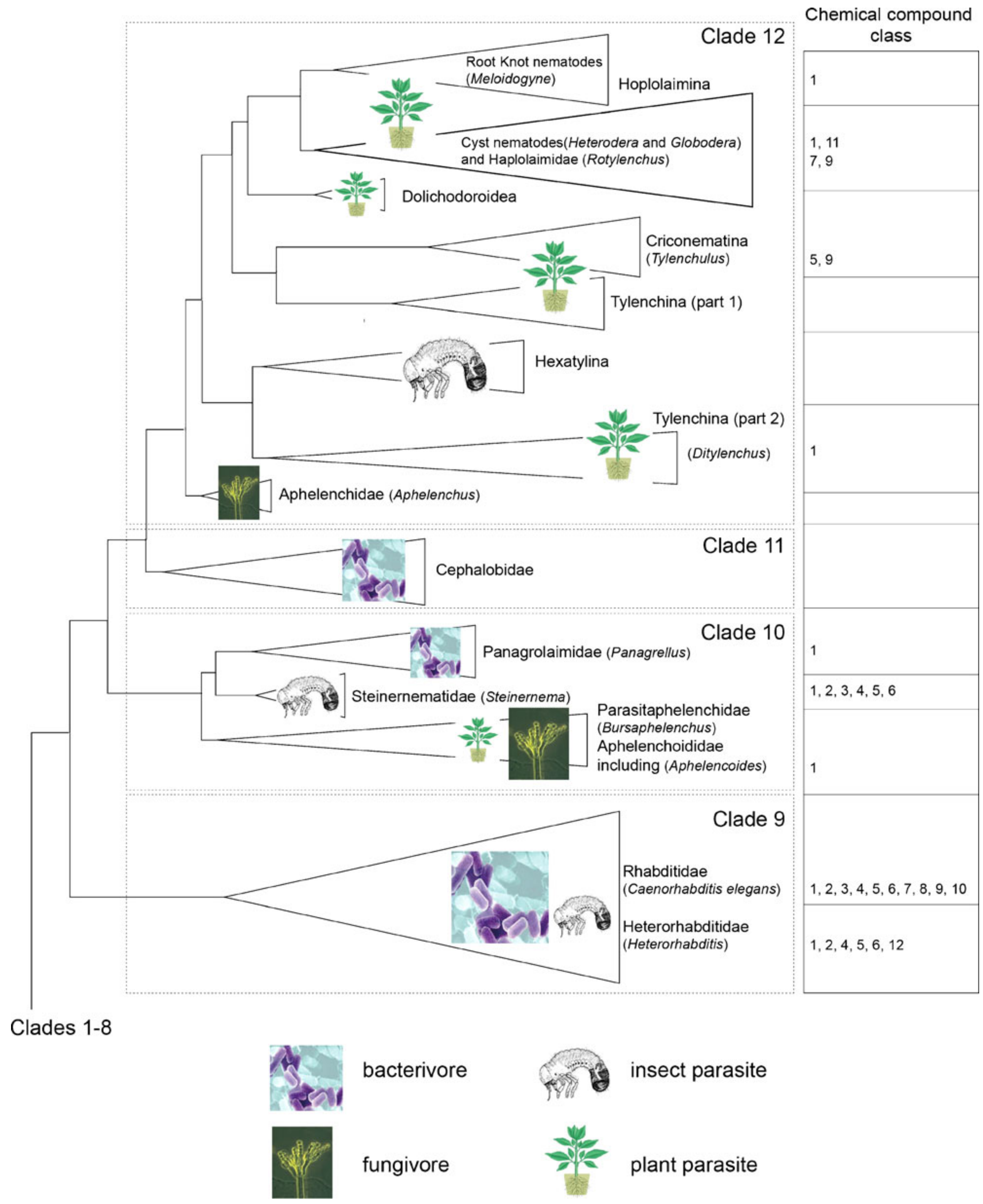
Indeed, besides CO_2, other recently discovered compounds involved in nematode attraction include plant-produced sesquiterpene molecules that can trigger attraction for phylogenetically and functionally different nematodes. Ali et al. (2011) showed that insect-induced citrus root chemicals (geijerene and pregeijerene) can attract the phytopathogenic nematode *T. semipenetrans*, as well as entomopathogenic nematodes *S. carpocapsae*, *S. riobrave*, and *H. indica*. Other compounds found to be triggering chemotaxis in various nematodes include various ions, salts, and amino-acids, again arguing for conserved chemo-sensory machinery across nematode species.

Given this striking conservatism in nematode sensory behavioral responses, can we still expect the evolution of the ability to sense particular chemical compounds in the soil? High levels of specificity would be strongly suggestive of such a relationship. For example, four closely related marine bacteriophagous nematodes have partially overlapping microhabitat preferences. These nematodes were found to have species-specific differences in their responses to three different strains of bacteria. This suggests that the least some level of food specialization may have occurred in conjunction with a specialized chemotaxis response (Moens et al., 1999).

Fig. 1 Evolution of chemotaxis in nematodes. Shown is the schematic SSU rDNA-based phylogenic relationship between nematodes belonging to Clades 9–12 (based on Holterman et al., 2006). *Right table* shows identity of chemical compounds that have been associated with nematode attraction toward odor sources. 1) atmospheric gas (CO_2), 2) alcohols, 3) ketones, 4) organic acids, 5) terpenoids, 6) thiazoles/pyrazidines, 7) cAMP, 8) esters, 9) ions, 10) amines, 11) amino acids, 12) aromatic compounds. See Table 1 for specific compounds. Overall, the figure shows the overwhelming presence of CO_2 as nematode attractant across different nematode taxa and feeding guilds. It also shows the paucity of data for many groups of nematodes (see text for details) ▶

Similar to other adaptive traits, different nematode chemical receptors may evolve if there is heritable variation in their production and effect, which in turn affects fitness. To our knowledge, measurement of genetic variation in nematode chemotaxis for specific compounds, and how this affects nematode fitness has not yet been attempted. We do, however, have evidence that different strains of nematodes can be recruited by different chemical compounds (Hiltpold et al., 2010; Moens et al., 1999). As various plant-parasitic nematode strains can differentially infect a given host plant, recognition and attraction might indeed be under selection (Perry et al., 2009). Hiltpold et al. (2010) have shown that only few cycles of selection are sufficient to increase *H. bacteriophora* attraction toward corn emitting (*E*)-β-caryophyllene. It is likely that strong directional selection of nematode attraction in corn fields will enhance the efficacy of entomopathogenic nematodes.

Still, there remains a gap in our interpretation of how insect-parasitic nematodes would have become sensitive to indirect cues of host location, such as herbivore induced plant volatiles. Answers may be provided when considering the life histories of closely related nematode taxa, along with their associated bacteria. For example, the insect-parasitic nematode genus *Heterorhabditis* most closely resembles a genus of marine nematodes, *Pellioditis* (Dougherty and Nigon, 1949). Species from *Pellioditis* are selective bacterial feeders that occur in intertidal and coastal regions (Poinar, 1993). There is evidence that the heterorhabditids evolved in a costal habitat from free-living microbiotrophic marine nematodes (Hara et al., 1991; Poinar, 1993). The bioluminescent bacterium that is responsible for the pathogenic effects of *Heterorhabdtis* on invertebrates is *Photorhabdus*. These bacteria are believed to have originated from a marine shore habitat, where there are many reports of living and dead marine invertebrates containing luminescent bacteria (Harvey, 1952). *Pellioditis marina*, a candidate for a pelloiditid that could have evolved into an insect-parasitic heterorhabditid, can survive on a luminescent bacteria (Tietjen et al., 1970). This may reveal a scenario that could permit a free-living bacterial feeder like *P. marina* to have evolved into an insect-parasitic nematode, where an injective juvenile came in contact with and retained bacteria



lethal to invertebrates. This early heterorhabditid need only to parasitize a littoral and beach dwelling crustacean, and a shift from crustacean to an insect would not have been difficult. Possible hosts would be root-feeding weevils (*Otiorhynchus* spp., Curculionidae) that are found along seacoasts on the roots of beach grasses (e.g., *Ammophila* *arenaria* or seashore wormwood, *Artemisia maritima*) or scarabid larvae of *Aegialia arenaria* (Scarabidae) along coastal dwelling and beach grass root feeders (Von Lengerken, 1929). Interestingly, scarabs and curculionids currently are known to be among the most susceptible soil insects to *Heterorhabditis* nematodes (Poinar and Georgis, 1990). If sensitivity to a volatile signal is as inheritable as demonstrated by studies of Hiltpold et al. (2010), entomopathogenic nematode sensitivity to herbivore induced plant volatiles becomes likely. Future work could evaluate this potential relationship by examining herbivore-induced compounds released by plant roots in sandy coastal regions, along with entomopathogenic nematode bioassays.

Ecological Impacts of Root Exudates

Different scenarios of root-exuded allomones to benefit overall plant fitness can be envisaged. For example, 1) plants can indirectly benefit from emissions of kairomones that attract bacterial or fungal feeders, which in turn can benefit plants by stimulating microbial community turnover and organic matter recycling (e.g., Luscher et al., 2004; Chapman et al., 2006). 2) Plants can emit molecules that can be defensive towards antagonists, such as the plant-parasitic nematodes (direct defense). For example, the roots of the french marigolds (*Tagetes patula* and *T. erecta*) contain α-terthienyl and other derivates of bithienyl, both of which can inhibit populations of *Meloidogyne* and *Pratylenchus* (Giebel, 1982). Roots of nematode-resistant banana plants were found to contain high levels of flavonoids, dopamine, caffeic esters, and ferrulic acids (Valette et al., 1998). Ferrulic acid molecules bound to cell walls of banana plants then were speculated to reduce the activity of cell wall-degrading enzymes in *Radopholus similis* nematodes (Wuyts et al., 2007). 3) Exudation of damaged roots can attract entomopathogenic nematodes to their arthropod hosts (indirect defenses). Based on evidence gathered here, different scenarios of root-exuded allomones to benefit overall plant fitness can be envisaged. For example, the common milkweed *Asclepias syriaca* is generally fed by the specialist root herbivore larvae of the cerambycid beetle *Tetraopes tetraophthalmus*. Emissions of volatile organic compounds by common milkweed in the soil can increase after insect damage. In lab experiments, this increased emission was correlated with increased entomopathogenic nematodes *H. bacteriophora* attraction. Subsequent field trials demonstrated that soil inoculation of entomopathogenic nematodes benefitted the plants by restoring plant biomass to control levels (Rasmann et al., 2011b). This, with previous work on bush lupine (Strong et al., 1996, 1999), is probably the best evidence of a natural subterranean trophic cascade that may result into enhanced plant performance. Whether or not this is correlated with higher levels of particular volatile emissions has not been assessed. Roots of *A. syriaca* plants emit a very complex mixture of >30 compounds of which only few are described as being in the terpene family (Rasmann et al., 2011b). Such a complex blend by itself

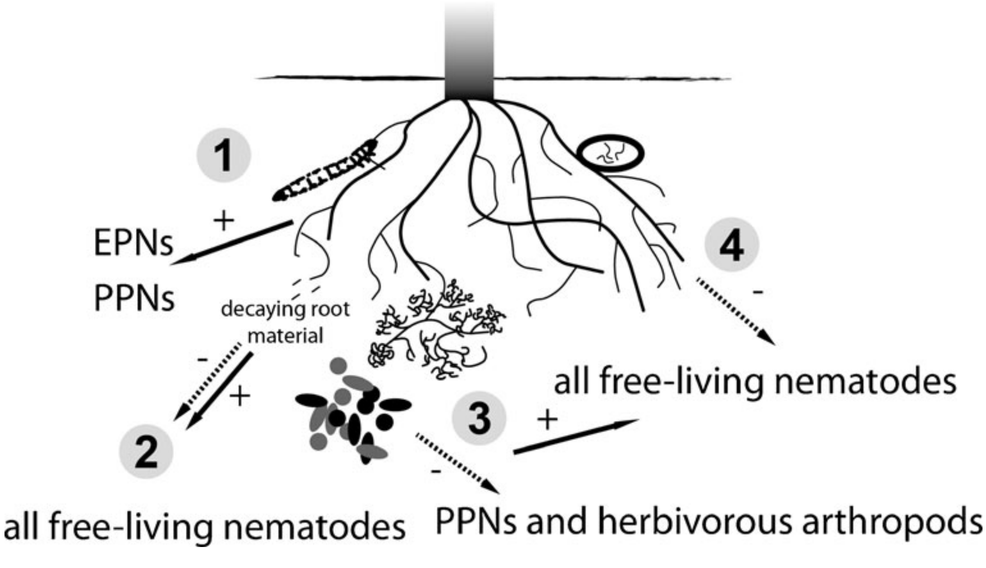


Fig. 2 Belowground plant chemically-derived nematode community structuring. Because of generalized and widespread detection of similar compounds across different nematode groups as shown in Fig. 1, we propose that 1) herbivorous arthropods or plant-parasitic nematodes (PPNs) can induce plants to release chemical organic compounds in the soil matrix, which can attract other herbivores (PPNs) as well as entomopathogenic nematodes (EPNs). 2) Root leachates and root-based detritus can become information cues for all free living nematodes in the rhizosphere. 3) Root symbiotic fungi and bacteria can stimulate root respiration (CO_2) and exudation to attract plant-parasitic nematodes as well as root-feeding arthropods. Fungi and bacteria not directly associated with roots by living in the rhizosphere can increase CO_2 levels to attract free-living soil nematodes. 4) Plants can counteract nematode attack by producing repelling compounds, which can simultaneously repel other free-living nematodes

already impedes the assessment of which particular compounds are responsible for the attraction. A problem similar to one found in above-ground systems (Hare, 2011), where the emerging picture is that volatile production in plants is the result of diffuse selection due to multiple players interacting with the plant.

The emission of organic molecules can have unintended effects on non-target organisms in addition to nematodes. For example, increased CO2 levels or other exudates have been shown to attract herbivorous arthropods, which can further decrease plant fitness (see Johnson and Nielsen, 2012, this issue). Therefore, nematode-induced changes in soil chemical characteristics may contribute to the structuring of specific communities around roots. These complex interactions may limit the development of optimal soil management practices. For example, the insect herbivore-induced emissions of terpenes by citrus plants have been shown to attract entomopathogenic nematodes (Ali et al., 2010; 2011). These compounds also may attract plant-parasitic nematodes *T. semipenetrans* (Ali et al., 2011). Therefore, unless rootstocks are not otherwise resistant to *T. semipenetrans*, this co-attraction may hamper the exploitation of citrus-induced volatile emission in biological control strategies that target the root weevil *Diaprepes abbreviates*. In Fig. 2 we have outlined possible direct- and indirect chemically-mediated effects on different nematode feeding guilds. Undoubtedly, future work is needed to complement the paucity of literature on the exact nature of compounds driving nematode foraging behavior.

Conclusions

Nematodes from different feeding guilds can ‘smell’ and ‘taste’ a variety of diverse compounds in soil. The sensatory capacity of different nematode feeding guilds is remarkably similar, and there appears to be a key role for some general compounds, such as CO_2, to be ubiquitous nematode attractants. Plant-borne soil chemical signatures can attract nematodes, thus structuring nematode communities in the rhizosphere. Different nematode species will in turn impose specific selective pressure on plants to produce a unique blend of chemical exudates. Of course, this only plays a role in wild plants that are not under artificial selection by plant breeders. The fitness benefits for plants to produce specific root exudates in soil is then the net outcome of diffuse co-evolution imposed by all soil organism in the rhizosphere, including nematodes from all trophic levels (Fig. 2).

The relative simple laboratory settings in which most bioassays described above were done undoubtedly have produced a highly simplified version of the complex chemical profile of natural soils, where thousands of similar molecules co-exist. Interestingly, however, chemical complexity seems to facilitate nematode foraging behavior. A recent report shows that CO_2 interacts synergistically with (*E*)-b-caryophyllene and dimethyl disulfide to increase *H. megidis* nematode attraction (Turlings et al., 2012). Future work should, therefore, aim at measuring single but also interactive effects of organic molecules that drive nematode behavior. Chemical characterization of agricultural soils might be a better starting point, not only for applied reasons of improving biological control of crop pests, but also from the fundamental point of view of understanding ecological mechanisms driving nematode foraging behavior. However, complementary studies in natural soils are needed in order to understand evolutionary mechanisms that drive nematode foraging behavior. For example, most of the volatile and non-volatile cues involved in belowground defense and resistance against herbivores remain unknown. Understanding more of these complex mechanisms that drive plant-nematode interactions would not only allow a better understanding of ecological interactions in the rhizosphere, but also offer ecologically sound alternatives in pest management in agricultural systems, such as breeding more attractive plants, intercropping attractive pest-resistant plants, or genetically modify crop plants for increased resistance (see Hiltpold and Turlings, 2012, this issue).

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Induced Immunity Against Belowground Insect Herbivores-Activation of Defenses in the Absence of a Jasmonate Burst

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Abstract Roots respond dynamically to belowground herbivore attack. Yet, little is known about the mechanisms and ecological consequences of these responses. Do roots behave the same way as leaves, or do the paradigms derived from aboveground research need to be rewritten? This is the central question that we tackle in this article. To this end, we review the current literature on induced root defenses and present a number of experiments on the interaction between the root herbivore *Diabrotica virgifera* and its natural host, maize. Currently, the literature provides no clear evidence that plants can recognize root herbivores specifically. In maize, mild mechanical damage is sufficient to trigger a root volatile response comparable to *D. virgifera* induction. Interestingly, the jasmonate (JA) burst, a highly conserved signaling event following leaf attack, is consistently attenuated in the roots across plant species, from wild tobacco to Arabidopsis. In accordance, we found only a weak JA response in *D. virgifera* attacked maize roots. Despite this reduction in JA-signaling, roots of many plants start producing a distinct suite of secondary metabolites upon attack and reconfigure their primary metabolism. We, therefore, postulate the existence of additional, unknown signals that govern induced root responses in the absence of a jasmonate burst. Surprisingly, despite the high phenotypic plasticity of plant roots, evidence for herbivore-induced resistance below ground is virtually absent from the literature. We propose that other defensive mechanisms, including resource reallocation and compensatory growth, may be more important to improve plant immunity below ground.

Keywords *Diabrotica virgifera* · *Zea mays* · Jasmonic acid · Induced resistance · Root herbivory · Plant defenses · Plant tolerance · Plant immunity

The Root Immune System

Plants possess an inducible immune system that helps them to cope with pathogens, nematodes, and arthropod herbivores. The functional components of this system above ground are well understood, and the current paradigm distinguishes at least four different steps from recognition to response: First, arthropod herbivores are perceived by plants via elicitors, also called herbivore and damage associated molecular patterns (HAMPs and DAMPs) (Felton and Tumlinson 2008), and wounding events. Second, a regulatory cascade is triggered, with jasmonates (JA) as central signaling components (Koo and Howe 2009). Third, both primary and secondary metabolisms are reprogrammed (Berenbaum and Zangerl 2008; Schwachtje and Baldwin 2008), resulting in plant phenotypical changes. Fourth and as a final result, plants become immune against the attacker via increased resistance and/or tolerance (Nunez-Farfan et al. 2007).

An overwhelming portion of the evidence supporting these central paradigms comes from studies above ground (van Dam 2009). However, to what extent are they valid for belowground plant parts? This question is particularly important, given the fact that arthropod herbivores of at least 25 insect families feed below ground, including many

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important agricultural pests (Hunter 2001). Root herbivores can have a strong impact on plant fitness (Blossey and Hunt-Joshi 2003), and it can be expected that, just as the leaves do, plant roots should possess an inducible immune system to defend themselves. Especially the combination of low probability of attack and high potential fitness cost should have favored the evolution of inducibility below ground (Zangerl and Rutledge 1996). Yet, despite an increasing number of studies that document dynamic root responses following insect attack (Puthoff and Smigocki 2006; van Dam and Raaijmakers 2006; Hiltpold et al. 2011), few attempts have been made to understand the mechanistic basis and adaptive value of induced root responses.

Roots, since their appearance in the first land plants 400 million years ago (Raven and Edwards 2001), are highly specialized structures that differ both morphologically as well as physiologically from their aboveground counterparts: Instead of chloroplasts for example, which play a central role not only in photosynthesis but also in defensive processes (Howe and Browse 2001), roots possess leucoplasts (Itoh and Fujiwara 2010), which are not pigmented and can serve as storage organelles. Also, instead of extracuticular barriers like waxes and trichomes (Valkama et al. 2005), roots interact with their environment directly via the apoplastic space that is separated from the vascular system by casparian strips (Waisel et al. 2002). From a resource perspective, roots have direct access to the major nutrients and water, but need to import assimilated carbon from the leaves. This high degree of differentiation makes it likely that the root immune system may have a mechanistic underpinning different from leaves (van Dam 2009).

In this paper, we combine three approaches to test the central paradigms of plant immunity below ground. First, we review the available literature on root immunity by using a comparative approach with aboveground studies. Second, we use the current understanding of general root physiology to predict specific differences in induction mechanisms. Third, we present a series of experiments on the reaction of maize plants to infestation by larvae of the specialist root feeder *Diabrotica virgifera virgifera*. Based on these three approaches, we present a series of general patterns and testable hypotheses about the mechanisms and consequences of herbivore-induced defenses below ground.

Do Roots Perceive Herbivores?

To be able to respond appropriately, plants have to recognize that they are under attack. Aboveground attackers are recognized by a series of associated molecular patterns. First, the wounds inflicted by chewing mouth-parts trigger the release and oxidation of otherwise contained and compartmentalized molecules, which then act as elicitors of defensive reactions (Ryan 2000; Huffaker et al. 2011). This process is also called damaged self-recognition (Heil 2009; Koo and Howe 2009), and the respective compounds are referred to as damage associated molecular patterns (DAMPS) (Boller and Felix 2009). Second, specific compounds in the saliva of insects, so called herbivore-associated molecular patterns (HAMPs) are recognized by the plant and boost the wound-induced immune response (Felton and Tumlinson 2008). A number of HAMPs have been structurally characterized for chewing herbivores (Alborn et al. 1997; Schmelz et al. 2007), and resistance genes that encode for receptor-like proteins have been discovered to mediate immunity against hemipteran phloem-feeders (Dogimont et al. 2010), which, taken together, leave little doubt that plants possess powerful and specific recognition systems to detect leaf-feeding herbivores (Erb et al. 2012). However, to what extent can plants recognize root attackers?

Several studies document that root herbivore attack induces pronounced defensive reactions below ground (van Dam 2009), which demonstrates that roots can perceive this type of biotic stress in some form. For phytopathogenic nematodes, the existence of resistance (R) genes hints at specific perception (Milligan et al. 1998). However, for insect herbivores, it is much less clear if roots have evolved the capacity to specifically recognize them, or if the measured responses are non-specific consequences of tissue rupturing. In maize, feeding by the root herbivore *Diabrotica virgifera virgifera* induces the emission of the sesquiterpene *(E)*-β-caryophyllene, which can be used by entomopathogenic nematodes to locate their host. Contrary to herbivore attack, “stabbing” the roots with a cork-borer did not result in the attraction of entomopatogenic nematodes (Rasmann et al. 2005). Similarly, mechanical wounding was not enough to elicit attraction of entompathogenic nematodes to citrus trees (Ali et al. 2010). Additionally, different root herbivores have been found to elicit different quantities of the *(E)*-β-caryophyllene (Rasmann and Turlings 2008), and a number of other studies report on differential responses of roots to wounding and herbivory (Blossey and Hunt-Joshi 2003). Until now, it is unclear if the observed differences are due to herbivore-specific recognition or to different degrees of wounding (Blossey and Hunt-Joshi 2003; Rasmann and Turlings 2008). In a microarray study, we found that wounding roots with a knife is enough to regulate 80% of the maize transcripts that are responsive to *D. virgifera* (Erb 2009), suggesting that cues associated with mechanical damage dominate recognition in herbivore-induced root responses. Testing this hypothesis will require further targeted experiments. Comparing wound-induced patterns with and without the application of insect oral secretions to the roots or using herbivores with ablated salivary glands (Musser et al. 2002) would help to answer the question whether plants use HAMPS to specifically recognize herbivores below ground. Another important aspect in this context is a role for microorganisms, which may invade the wound-sites and trigger defensive reactions. Experiments under sterile conditions may help to evaluate their contribution as eliciting factors below ground.

Overall, from an evolutionary perspective, there may be less selection pressure for roots to use specific molecular patterns to recognize herbivores than for leaves: The above-ground parts are constantly damaged by wind, wind-transported particles, rain, and heavier animals, which may favor specific recognition mechanisms as a way of reducing costs of “false alarm”. Roots, however, may experience fewer abiotic mechanical damage events, and, therefore, wounding itself may be enough to reliably indicate the presence of an herbivore. From a physiological perspective, the capacity of plants to detect HAMPs below ground will depend on the presence of specific extra- or intracellular receptors in the roots. Plant roots can sense mechanical resistance (Hofmann 2009), nutrients (Schachtman and Shin 2007), and water (Kobayashi et al. 2007) as well as beneficial and pathogenic microorganisms (Mathesius and Noorden 2011), and, therefore, are likely to have a considerable array of environmental sensors and PAMP receptors at their disposition. Environmental sensing below ground is further facilitated by the enormous contact surface between roots and the surrounding matrix: At a macro scale, fine roots maximize contact surface, and at a micro-scale, the apoplastic space multiplies this surface many fold (Waisel et al. 2002). Thus, given sufficient selection pressure by herbivores, plant roots could very quickly have evolved the capacity to recognize HAMPS. The recognition of DAMPS on the other hand is a general mechanism for stress perception, as it involves compounds that are liberated from the plant following attack (Heil 2009). From a physiological perspective, the “damaged self” below ground is likely to be strikingly different from what is known in the leaves: Because roots have a distinct chemical composition, including different membrane lipids, sugars, amino acids, and secondary metabolites, wounding by herbivores could also result in differential DAMP release. It will be interesting to assess whether roots have evolved to recognize their own “damaged self” or whether the same DAMPs trigger wound-reactions above and below ground.

To understand in detail whether mechanical wounding is enough to trigger root defenses in maize, we performed an experiment with 4-day-old maize seedlings, which show a similar volatile response to herbivore attack as older seedlings (personal observations). The seedlings were either pierced 4 times with a fine needle or infested with 12 *D. virgifera* larvae. We then determined the release of (*E*)-β-caryophyllene, the major herbivore-induced volatile in the roots, every 2 hours over a period of 18 hours *in vivo* using solid phase microextraction (SPME) as described (Robert et al. 2012). As shown in Fig. 1, wounding the roots was sufficient to trigger a burst of (*E*)-β-caryophyllene, which was only matched in intensity after 10 hours of continuous *D. virgifera* feeding. From this experiment, it can be concluded that herbivore-derived cues are not strictly required to trigger (*E*)-β-caryophyllene emission in maize roots, at least at this young seedling stage. Relatively mild mechanical damage is, in fact, enough to prompt a quick and robust response that is similar to herbivore feeding, suggesting low specificity of recognition. Further experiments will be necessary to exclude a potentiating effect of HAMPS from *D. virgifera* on induced defenses in maize roots.

Induced Root Signaling and the Elusive Role of Jasmonates

Following recognition by DAMPs and HAMPs in the leaves, plants start deploying intricate signaling cascades. Quick membrane depolarization by Ca^{2+} influx is followed by the generation of reactive oxygen species (ROS), the induction of mitogen activated protein kinases (MAPKs) and WRKY transcription factors, and, eventually, a pronounced increase in phytohormones (Wu and Baldwin 2009). The burst in fatty acid derived oxidative products, so called oxilipins, with jasmonic acid isoleucine (JA-Ile) as the main active jasmonate, is widely accepted to be the master regulator of induced responses against chewing herbivores (Howe and Jander 2008). However, a number of other phytohormones also mediate defensive responses, either by acting as modulators of the JA pathway, as is the case for ethylene (ET) (Onkokesung et al. 2010) and abscisic acid (ABA) (Bodenhausen and Reymond 2007; Erb et al. 2011b), or as independent inducers of distinct immune responses, as

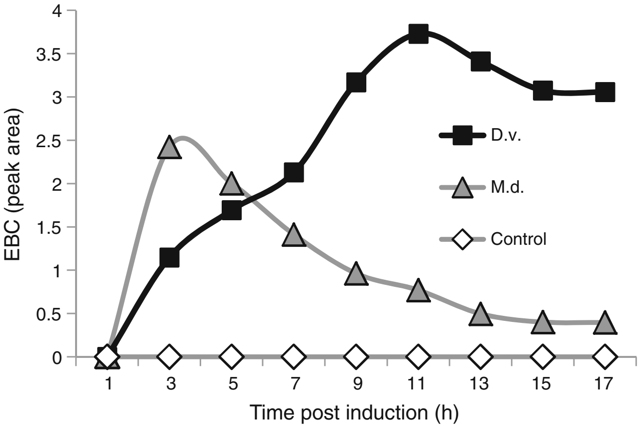

Fig. 1 *(E)*-β-caryophyllene is induced by mechanical damage and *Diabrotica virgifera* feeding. Quantities of *(E)*-β-caryophyllene (peak area/20 min) emitted from maize seedlings over time are shown. Seedlings were either untreated (Control, white diamonds), mechanically damaged with a needle (M.d., grey triangles), or infested with *D. virgifera* larvae (D.v., black squares). Amounts represent cumulative quantities from 3 equally treated seedlings

shown for salicylic acid (SA), a key player in resistance against hemipteran feeders (Gao et al. 2008). Hormones like cytokinins (CKs) and auxins that have formerly been associated with growth regulation are increasingly recognized as defense regulators as well (Erb et al. 2012). Does herbivory trigger similar signaling events below ground?

Several recent studies have measured the jasmonate response below ground following herbivore attack or mechanical injury. In *Medicago truncatula*, mechanical wounding during root harvest led to a modest increase in JA levels. Interestingly, the systemic JA response in the leaves upon root stress was stronger than the local response in the roots (Tretner et al. 2008). Similar effects were reported for *Arabidopsis thaliana* (McConn et al. 1997; Hasegawa et al. 2011). Maize roots were found to respond to herbivory by increasing their JA levels 2-fold (Erb et al. 2009a), which is modest compared to the pronounced JA response in the leaves following herbivory (Schmelz et al. 2003). Finally, in *Nicotiana attenuata*, a species that shows a pronounced wound-induced jasmonate burst above ground, wounding of the roots led to a modest 2-fold increase in JA levels (Bonaventure et al. 2011). Taken together, this is strong evidence that the JA-burst in plant roots generally is attenuated compared to the leaves (Fig. 2). Nevertheless, several studies point to a role for jasmonates in root defense: Exogenous application of JA or its methyl-ester MeJA increased the belowground production of ectosteroids in spinach (Schmelz et al. 1998), volatiles in maize (Erb et al. 2011a), glucosinolates in *Brassica spp*. (Pierre et al. 2012), nicotine in *Nicotiana spp*. (Baldwin 1989), a subset of sugar beet root maggot induced genes in *Beta vulgaris* (Puthoff and Smigocki 2007) and defense related transcripts in *Arabidopsis thaliana* (Hasegawa et al. 2011).

We propose three hypotheses that may reconcile the seemingly contrasting results that i) root defenses are JA-

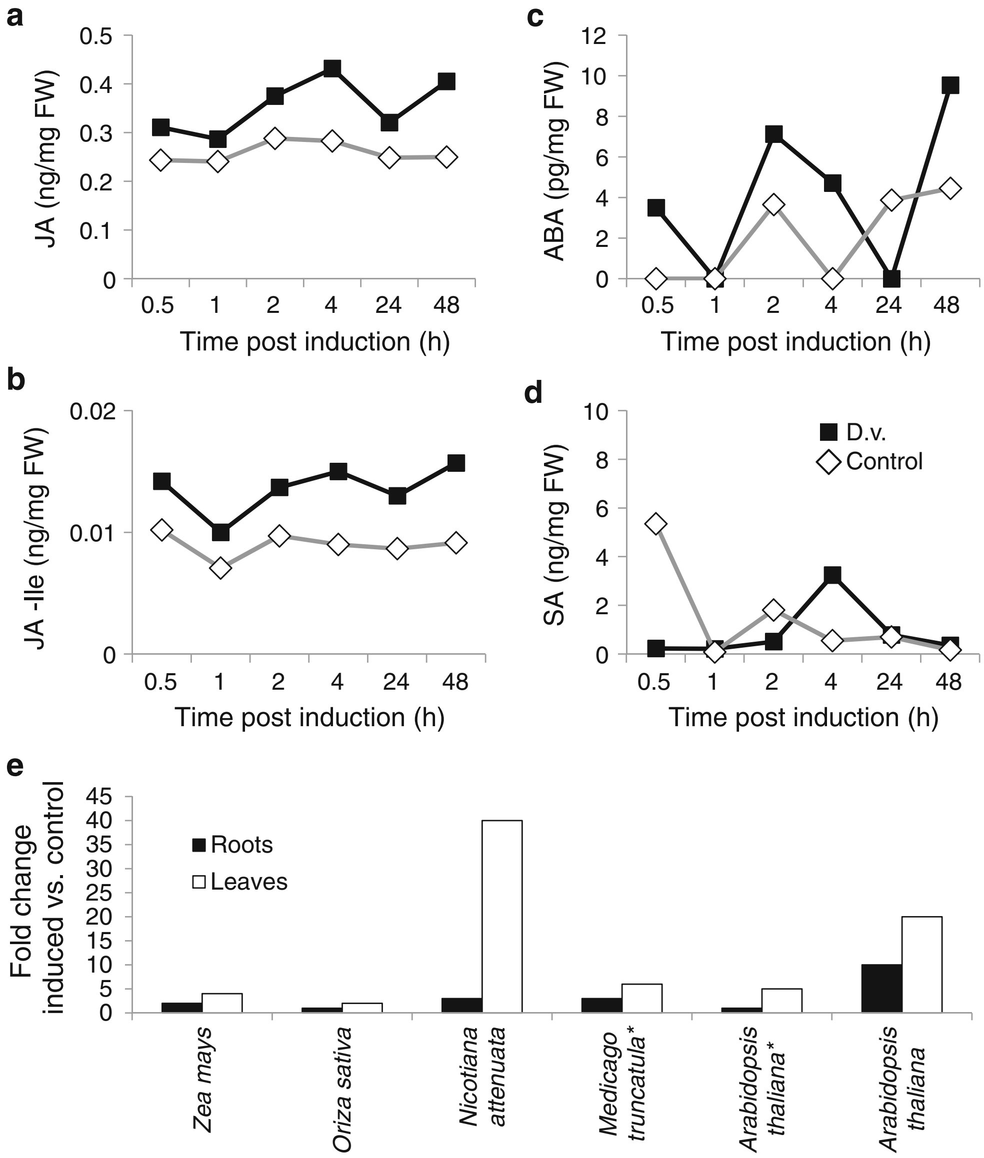

Fig. 2 Induction of phytohormones in the roots. **A**–**D**: Average quantities (ng/mg FW) of phytohormones produced by maize roots at different time points after onset of *Diabrotica virgifera* attack. (**A**) Jasmonic acid (JA), (**B**) jasmonic acid isoleucine conjugate (JA-Ile), (**C**) salicylic acid (SA), and (**D**) abscisic acid (ABA) are shown for control plants (Control, white diamonds) and *D. virgifera* infested plants (D.v., black squares). Values correspond to average values from 6 equally treated plants. E: Average fold change of JA levels in the roots and leaves of different plants following local induction. Asterisk indicates datasets that measured JA in roots and leaves following root induction only. For references, see text

inducible, but that ii) roots produce little JA in response to herbivore attack. First, the sensitivity of roots to jasmonates may be higher, making it possible that even small changes in jasmonates are sufficient to trigger defensive reactions. Second, JA-derivatives other than JA-Ile may be the triggering signals in the roots. Third, the activation of JA-responsive elements may occur indirectly via other signals. Until now, little is known about a possible involvement of other phytohormones in induced root immunity. In our studies, we did not find any induction of ABA or SA in *D. virgifera* attacked maize roots (Erb et al. 2009a), despite the fact that ABA seems to be a herbivore-induced root-shoot signal (Erb et al. 2009b). Furthermore, the application of SA to broccoli roots could not mimic the induction of glucosinolates by *Delia radicum* (Pierre et al. 2012). The potential of SA and ET to modulate root-herbivore induced responses has been discussed by Puthoff and Smigocki (2007), who found several root maggot induced genes in *Beta vulgaris* to be responsive to these hormones. The involvement of growth hormones like auxins and CKs in induced-responses below ground has not been investigated. However, given the fact that i) belowground structures exhibit extensive regrowth and tolerance patterns following herbivory (Rubio and Lynch 2007; Poveda et al. 2010), and that ii) auxin and CKs regulate root growth patterns and branching (Aloni et al. 2006), we hypothesize that these hormones may play an important role in induced responses below ground.

From a physiological perspective, roots can employ the same signaling molecules as leaves. Major phytohormones, for example, can be detected in roots (Erb et al. 2009a). However, while the molecular vocabulary of roots and shoots may be the same, they speak a very different language: Auxin for example inhibits lateral branching above ground, but promotes root elongation below ground (Benjamins and Scheres 2008). Abscisic acid is important for stomatal regulation in leaves and regulates root growth (Cutler et al. 2010). Therefore, it can be expected that the signaling processes involved in induced root immunity will also differ from the leaves. Furthermore, the capacity of roots to synthesize specific signals following herbivory may be different from the leaves: The JA precursor linolenic acid, for example, is much less abundant in the roots than in the leaves. Instead, roots contain higher concentrations of linoleic acid (Li et al. 2003), the precursor of dihydro-JA, one of many other jasmonates with biological activity (Blechert et al. 1995; Erb and Glauser 2010). It is tempting to speculate that other jasmonates than JA-Ile may be important for defense signaling in the roots.

We measured the changes in several phytohormones in the roots of 12-day-old maize plants following *D. virgifera* attack as described (Hiltpold et al. 2011) using a previously described protocol (Erb et al. 2011b). The results show that both JA and JA-Ile increased locally by only about 50% 30 minutes after *D. virgifera* attack. The levels then remained stable over the two days of infestation (Fig. 2). On the other hand, SA and ABA did not show any clear induction pattern. The phytohormone responses are in strong contrast to the volatile production, measured in the same samples: *(E)*-β-caryophyllene emissions start about 4 hours after onset of feeding, and then increase exponentially (Hiltpold et al. 2011). The hormonal response of maize roots measured here adds to the growing evidence that roots do not show a characteristic JA-burst upon elicitation (Fig. 2). Furthermore, while volatile production and JA-elicitation are strongly correlated in the leaves (Schmelz et al. 2003), there is an obvious disconnect between maize root JA induction and *(E)*-β-caryophyllene, indicating that signals other than JA may be able to trigger the activation of sesquiterpene biosynthesis. Investigation of the regulatory mechanisms of root induction should therefore be a priority of future research on belowground defenses.

Induced Chemical and Phenotypical Changes in Roots

In the leaves, herbivore-induced defense signals trigger a variety of phenotypical changes. Typically, the reprogramming involves i) Induction of volatile organic compounds (Hare 2011), ii) induction of non-volatile secondary metabolites (Glauser et al. 2011), iii) induction of defensive proteins (Zhu-Salzman et al. 2008), iv) redirection of assimilate fluxes (Schwachtje and Baldwin 2008), and v) long term morphological changes (Strauss and Agrawal 1999; Traw and Dawson 2002). Does root herbivory result in similar phenotypical changes?

Recent studies show that attack by root-feeding insects triggers distinct phenotypical changes below ground that rival their aboveground counterparts in both diversity and magnitude. Several recent reviews have discussed induced phytochemical responses in detail (Kaplan et al. 2008b; Rasmann and Agrawal 2008; van Dam 2009), and a number of remarkable trends are emerging. First, roots produce different inducible secondary metabolites from the leaves. An illustrative example is the release of volatile compounds of herbivore attacked maize plants: Leaves of herbivore-attacked plants produce over 30 different compounds in response to real or simulated herbivory, including green leaf volatiles (GLVs), aromatic compounds, homo, mono, and sesquiterpenes. Two sesquiterpenes, (*E*)-β-farnesene and (*E*)-α-bergamotene, are dominating the induced leaf-blend (Erb et al. 2010). Attacked maize roots on the other hand produce only a few sesquiterpenes and traces of the aromatic compound indole (Hiltpold et al. 2011). The sesquiterpene (*E*)-β-caryophyllene dominates the root blend, while (*E*)-β-farnesene and (*E*)-α-bergamotene cannot be detected at all. Tissue specific patterns also have been observed for herbivore induced volatiles of Swingle (*Citrus paradisi* x *Poncirus trifoliata*) (Ali et al. 2011). Furthermore, non-volatile secondary metabolites also are produced differentially in the roots. Alkaloids are a prominent example in this context: Although they are induced by leaf-herbivory and accumulate above ground, their biosynthesis often occurs exclusively in the roots (Ziegler and Facchini 2008). Additionally, Brassica plants can produce specific root glucosinolates against nematode herbivores (van Dam et al. 2009). Conversely, insecticidal phenolics such as maysin are produced only in the leaves and silks of maize plants (Nuessly et al. 2007), but not in the roots (Robert et al. 2012). Roots are thus unique bioreactors, and the cocktails of secondary compounds that they produce following herbivore attack are highly distinct.

A second emerging trend is that even compounds that are produced by both leaves and roots may differ in their inducibility between the two tissues. The insecticidal benzoxazinoid HDMBOA-Glc for example is highly inducible in maize leaves (Glauser et al. 2011), but constitutively produced below ground (Robert et al. 2012). The furanocoumarin xanthotoxin, on the other hand, is produced in constitutively high levels in the leaves of wild parsnip, but inducible in the roots (Zangerl and Rutledge 1996). These findings underscore the fact that the root secondary metabolism is specifically regulated.

Most inducible secondary metabolites are activated in a quantitative manner by JA signaling (Howe and Jander 2008), but root attack leads to an attenuated JA burst compared to the leaves (Fig. 2). We therefore reason that, if jasmonates are the predominant signals regulating root responses, the wound- and herbivore-induced production of defensive compounds in the roots should generally be reduced compared to the leaves. Surprisingly however, a recent meta-analysis found that root secondary metabolites are, overall, as inducible as their leaf-counterparts (Kaplan et al. 2008b). Because this study excluded mechanical damage treatments, but included pathogens and nematodes, we recompiled data from the literature focusing on secondary metabolites that i) occur in the leaves and roots, and ii) have been measured in both leaves and roots after local induction by herbivores or mechanical damage. Apart from data from 9 published studies (Zangerl and Rutledge 1996; Schmelz et al. 1998; Bezemer et al. 2003, 2004; Soler et al. 2005; van Dam and Raaijmakers 2006; Rasmann et al. 2009; Glauser et al. 2011; Robert et al. 2012), we included unpublished data on hydroxycinnamoyl tyramines from maize (G. Marti, unpublished). Two principal trends become visible (Fig. 3): First, for many defensive metabolites, leaves are indeed more inducible than roots (5 out of 7). Second, some specific secondary metabolites, namely phytoecdysteroids in spinach (Schmelz et al. 1998) and xanthotoxins in wild parsnip (Zangerl and Rutledge 1996) are more inducible in the roots. It would be informative to measure the root JA-burst in these two plant species to see whether their higher inducibility below ground is correlated with a high JA responsiveness, or whether, just as for (*E*)-β-caryophyllene in maize, the existence of alternative signals has to be envisaged. It should be emphasized that some secondary compounds may be less inducible in the roots because they are already present in high constitutive amounts. This is for example the case for glucosinolates (van Dam et al. 2009) and benzoxazinoids (Robert et al. 2012).

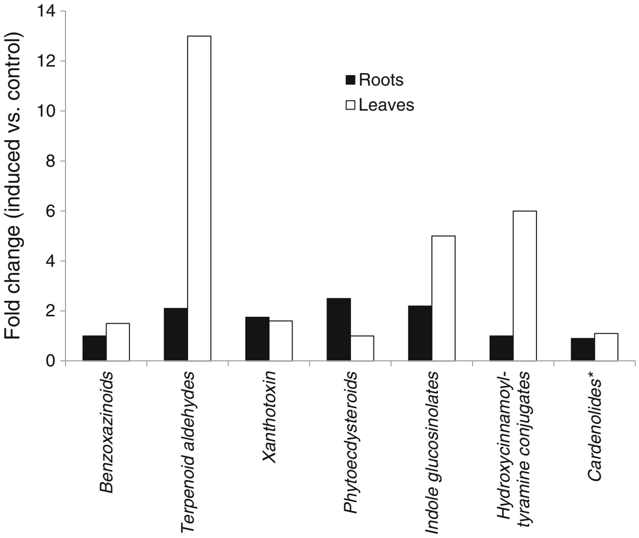

Fig. 3 Average fold change of secondary metabolites in the leaves and roots of locally induced plants. Induction was achieved by either mechanical damage or insect herbivory. *Induction of cardenolides was not significant. Data from Zangerl and Rutledge 1996; Schmelz et al. 1998; Bezemer et al. 2003, 2004; Soler et al. 2005; van Dam and Raaijmakers 2006; Rasmann et al. 2009; Glauser et al. 2011; Robert et al. 2012; G. Marti, unpublished

To get insight into the herbivore-induced changes of the root secondary metabolism of maize, we performed a metabolomics screen on *D. virgifera* induced roots of 12-day-old maize seedlings. The employed methodology was the same as published previously (Erb et al. 2011a; Glauser et al. 2011) and covered both volatile and non-volatile secondary metabolites. A similar experiment in maize leaves found a pronounced induction of several non-volatile secondary metabolites (Glauser et al. 2011). *Diabrotica virgifera* infestation led to clear changes in the root volatile profile, leading to a separation of controls and induced plants along the first principal component (PC) axis (Fig. 4). The only significant change was the induction of (*E*)-β-caryophyllene, as reported by previous studies (Rasmann et al. 2005). Surprisingly, however, we could not find any clear induction of non-volatile secondary metabolites in either positive or negative detection mode with UHPLC-QTOF-MS (Fig. 4). This is in contrast to the leaves (Glauser et al. 2011) and shows that the root secondary metabolome of maize is pronouncedly less responsive to herbivory

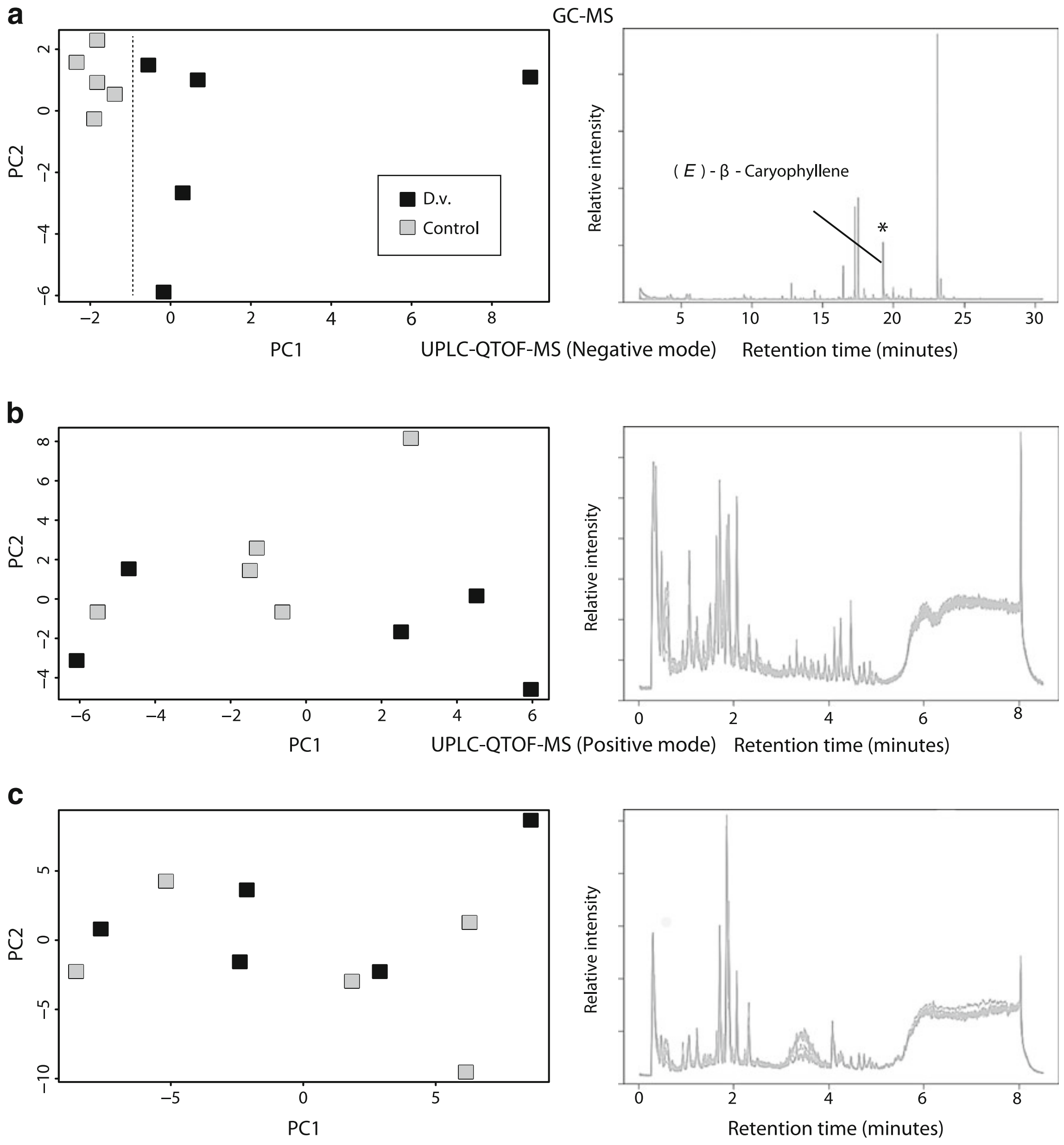

Fig. 4 Metabolomics screen of *Diabrotica virgifera* attacked maize roots. Results of principal component analyses (PCAs, left) and total ion chromatograms (TICs; right) for volatiles detected by GC-MS (**A**) and non-volatile metabolites detected with UHPLC-QTOF-MS in negative (**B**) and positive (**C**) ionization modes are shown. PCAs: Individual control samples (grey squares) and *D. virgifera* induced samples (*black bars*) are depicted. Compounds that were different between treatments $P>0.01$, fold change >1.5) are denoted with an asterisk in the TICs

than the leaf metabolome. The hypothesis that *D. virgifera*, as a specialist, may suppress root responses in maize is unlikely, as we found similar results for plants that were attacked by the generalist *D. balteata* (data not shown). This finding underscores the trend that roots are generally less inducible than leaves, but that they nevertheless produce some specific secondary metabolites following herbivore attack.

Compared to low-molecular weight secondary metabolites, less is known about the induction of defensive proteins in roots following herbivore attack. Maize lines with a leaf insect-inducible cysteine protease (Mir1-CP) were found to be resistant against *D. virgifera*, indicating that Mir1-CP may accumulate in attacked roots as well (Torrence et al. 2011). Chitinase activity was increased in *Diaprepes abbreviatus* infested citrus rootstocks (Mayer et al. 1995). We furthermore found that *D. virgifera* feeding induces the expression of several proteinase inhibitor genes (C.A.M. Robert, unpublished data), indicating that proteinase inhibitor activity may increase in infested roots. So far, both Mir1-CP and PI induction have been observed in both above- and belowground organs (Lopez et al. 2007; Erb et al. 2009a). Untargeted proteomic approaches will be necessary to find out whether roots express specific protein-based defenses against insects. As the cost for nitrogen acquisition may be lower below than above ground, while the inverse may be true for carbon, it is well possible that evolution may have favored nitrogen- over carbon-consuming defenses below ground (Erb et al. 2009c). Apart from nitrogen containing secondary metabolites like alkaloids, defensive proteins may thus be more important in the roots than in the leaves.

Can roots reallocate resources to other tissues in the case of an attack? A study on root-herbivore tolerant *Centaurea maculosa* plants suggests that this may indeed be the case: When attacked by the root boring *Agapeta zoegana*, *C. maculosa* allocated more nitrogen to the shoots, especially under low nitrogen supply (Newingham et al. 2007), a behavior which may help the plant sustain high photosynthetic activity for compensatory growth. Further indirect evidence for changes in resource allocation comes from a study on potato that showed an increase in non-attacked tuber weight following infestation by root herbivores (Poveda et al. 2010). The JA-application to one half of a tomato root system also leads to an increase in carbon allocation to the non-treated half (Henkes et al. 2008). Resource reallocation following leaf-attack has been attributed to changes in sink-source relationships: Herbivore-attacked tobacco plants increase invertase activity in the roots (Kaplan et al. 2008a), which may increase root sink strength for carbon. When the roots are under attack, this process could simply be reversed: A reduction of invertase activity would likely lead to increased allocation to the aboveground parts of the plant. How nitrogen reallocation may be achieved remains to be determined (Lalonde et al. 2004), but it is possible that an increase in photosynthetic activity following root herbivore attack leads to an increased demand in, and, consequently, transport of nitrogen to the leaves (Godfrey et al. 1993).

Tightly linked to resource reallocation are root growth responses and morphological changes following herbivore attack. Upon root damage, certain plants start growing new roots to replace the lost tissue. *Medicago sativa* plants attacked by the root feeding larvae of *Sitona hispidula* for example react with a pronounced regrowth response that can even result in overcompensation (Quinn and Hall 1992). A particularly interesting morphological response was found in white clover plants attacked by *S. lepidus*: Five days after infestation, the tissue density of the different roots had almost tripled (Care et al. 2000), which might be the result of additional lignification, which again could lead to tougher and more resistant roots (Johnson et al. 2010). Care and coauthors also note that clover genotypes with long fine roots suffered less from root herbivory than genotypes, with short, thick roots (Care et al. 2000). It is tempting to speculate that upon root herbivore attack, plants may change the structure of their root system to spread the risk of attack from a few main roots to many fine roots. New techniques for root visualization may help to unravel root morphological responses to belowground attack (Clark et al. 2011b). In this context, it is important to note that different belowground tissues can vary in their physiological properties and defensive status, and that resolving root defenses spatially is important to understand their effect on root herbivores (van Dam and Vrieling 1994; Robert et al. 2012).

Do Plants Use Alternative Strategies to Survive a Root Attack?

Evidence is accumulating that many leaf-responses improve plant immunity: Induced resistance, for instance, improved the fitness of wild radish and tobacco under high herbivore pressure (Agrawal 1998; Baldwin 1998). Application of extrafloral nectar as a means of attracting natural enemies increased growth rates and flower numbers of wild lima bean (Kost and Heil 2008). Activation of a carbon reallocation response by silencing a SNF1-related kinase delayed senescence and prolonged flowering in wild tobacco (Schwachtje et al. 2006). The testing for benefits of induced responses for the plant is crucial to understanding their adaptive value, and to distinguishing actual defenses from artifacts (Karban and Myers 1989). Do induced root responses improve plant immunity?

Despite an increasing number of studies on the topic, evidence for induced root resistance (i.e., the reduction of herbivore damage due to induced direct defenses) remains remarkably scarce. During our literature search, we found only one example where infestation by a belowground herbivore triggered an increase in root resistance against the same species: Vine weevils (*Otiorhynchus sulcatus*) feeding on raspberry plants that had previously been infested with conspecifics grew 19% less than larvae feeding on control plants (Clark et al. 2011a). In contrast, larvae of the onion root fly *Delia antiqua* were found to survive best on slightly damaged onion bulbs (Hausmann and Miller 1989), and *D. radicum* larvae tended to grow better on previously infested turnip plants (Pierre et al. 2012). In addition, our own experiments show that *D. virgifera* larvae perform better on roots of previously infested maize plants than on uninduced controls (Robert et al. in press). More experiments are needed to determine the prevalence of induced resistance in roots, but given the current literature, it seems that induced root resistance may not be as common as induced leaf resistance. Interestingly, many studies document that roots do have the potential to increase their resistance, given the right stimulus: The application of JA increases resistance of broccoli roots against *D. radicum* larvae (Pierre et al. 2012) and resistance of grapevine against the root feeding grape phylloxera *Daktulosphaira vitifoliae* (Omer et al. 2000). Furthermore, leaf herbivory induces systemic resistance in roots (Soler et al. 2007; Erb et al. 2011c; Torrence et al. 2011). This suggests that, while roots have the capacity to increase their direct defenses and resistance, root herbivory simply may not trigger the appropriate signaling events. A possible evolutionary explanation for the absence of induced root resistance may be that induced resistance *per se* provides little benefit to the plant. For example, many root herbivores are specialists and, therefore, potentially resistant to inducible defenses (Robert et al. 2012). Furthermore, because movement of root herbivores is restricted in the soil and because they often tunnel into larger roots, it may be more difficult for a plant to deter attackers by inducible defenses, a putative strategy above ground that may enable plants to gain a competitive advantage, as the neighboring plants will suffer from the emigrating herbivores (van Dam et al. 2000). Clearly, further research is required to understand whether induced responses to belowground herbivores can contribute to improved plant fitness, or whether other defensive strategies generally are more effective, and, consequently, favored by evolution.

One proposed alternative strategy to induced direct resistance is the release of herbivore-induced root volatiles, which may protect plants against belowground feeders by attracting natural enemies (see Hiltpold and Turlings, this issue). Maize plants attacked by *D. virgifera,* for example, release a sesquiterpene signal that is used by entomopathogenic nematodes to locate and kill the herbivore (Rasmann et al. 2005). Comparable effects have been found in citrus (Ali et al. 2010) and rapeseed (Ferry et al. 2007) The application of nematodes to *Asclepias syriaca* plants infested with larvae of the root-boring beetle *Tetraopes tetraophthalmus* prevented the loss of biomass to root herbivory (Rasmann et al. 2011), thus hinting at the possibility that attracting nematodes may be beneficial to the plant. However, just as in the aboveground case (Heil 2008), clear evidence that herbivore-induced root volatiles are indeed an indirect defense against herbivores is lacking. On the contrary, phytopathogenic nematodes (Ali et al. 2010) as well as herbivores themselves (Robert et al. in press) may use induced root signals to find host plants (see Rasmann et al., this issue). Until now, it remains unclear whether the defensive responses of roots are directly targeted at herbivores, or are deployed to avoid secondary infections by opportunistic pathogens (Kurtz et al. 2010), which are a constant threat below ground.

Compared to induced direct and indirect defenses, there is little doubt that induced tolerance responses to root herbivory in the form of rapid regrowth improve plant immunity. *Medicago sativa* plants that regrow root nodules to equal or higher numbers after denodulation by larvae of *S. hispidulus,* for example, do not suffer from a reduction of biomass (Quinn and Hall 1992), while *Trifolium repens* plants lose a significant amount of their nodules and, consequently, suffer negative consequences from *Sitona lepidus* attack (Murray et al. 2002). A locally adapted potato variety from the Columbian Andes even increases tuber production and aboveground biomass when attacked by the Guatemalan potato moth (*Tecia solanivora*) in low densities (Poveda et al. 2010), a reaction that implicitly increases plant fitness compared to potato varieties that fail to show this type of response. Finally, the capacity of maize plants to regrow roots following *D. virgifera* infestation can significantly reduce yield loss (Prischmann et al. 2007). It remains to be demonstrated whether other putative root-herbivore tolerance strategies such as changes in resource allocation (Newingham et al. 2007; Orians et al. 2011) or increased photosynthesis (Godfrey et al. 1993) can be linked to improved plant immunity. Overall, it appears that tolerance strategies may be more commonplace than induced resistance below ground.

Conclusions

Root defenses are special. The current literature provides ample evidence for this statement and indicates that roots: i) perceive herbivores differently; ii) use different signals to react to herbivore attack; iii) synthesize specific defensive compounds; and iv) may use distinct survival strategies to improve their immunity in the face of a herbivore threat. However, many questions remain open, and mechanistic aspects have not been tackled with the same rigor as above ground, thus making many of the above conclusions tentative. Understanding the mechanisms of induced root immunity will not only broaden our horizon on plant defensive strategies, but may eventually lead to novel, paradigm-shifting insights into the secret life of roots and belowground herbivores.

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REVIEW ARTICLE

Manipulation of Chemically Mediated Interactions in Agricultural Soils to Enhance the Control of Crop Pests and to Improve Crop Yield

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Abstract In most agro-ecosystems the organisms that feed on plant roots have an important impact on crop yield and can impose tremendous costs to farmers. Similar to aboveground pests, they rely on a broad range of chemical cues to locate their host plant. In their turn, plants have co-evolved a large arsenal of direct and indirect defense to face these attacks. For instance, insect herbivory induces the synthesis and release of specific volatile compounds in plants. These volatiles have been shown to be highly attractive to natural enemies of the herbivores, such as parasitoids, predators, or entomopathogenic nematodes. So far few of the key compounds mediating these so-called tritrophic interactions have been identified and only few genes and biochemical pathways responsible for the production of the emitted volatiles have been elucidated and described. Roots also exude chemicals that directly impact belowground herbivores by altering their behavior or development. Many of these compounds remain unknown, but the identification of, for instance, a key compound that triggers nematode egg hatching to some plant parasitic nematodes has great potential for application in crop protection. These advances in understanding the chemical emissions and their role in ecological signaling open novel ways to manipulate plant exudates in order to enhance their natural defense properties. The potential of this approach is discussed, and we identify several gaps in our knowledge and steps that need to be taken to arrive at ecologically sound strategies for belowground pest management.

Keywords Rhizosphere food web · Root pest control · Soil signaling · Root volatile · Crop protection · Belowground plant defense · Nematode

Introduction—Belowground Herbivory and Plant Defense

For decades, plants roots have been mainly considered as defenseless victims of soil-dwelling pests and a passive sink for leaf-produced photoassimilates. However, an increasing number of recent studies emphasize that, instead of being idle victims, roots play a major role in defending themselves and aboveground tissues, and in shaping their surrounding habitat via production and exudation of organic chemicals (Bais et al., 2006; Erb et al., 2009; van Dam, 2009). In fact, a large number of soil organisms have been shown to rely on root exudates as a carbon source (Walker et al., 2003), dramatically diverging from the formal assumption that the soil fauna is largely dependent on aboveground litter for carbon (Huhta, 2006). Beside anchoring the plant in soil and being the principal channel of nutrient transfer from the soil to the aboveground tissues of the plants and further trophic levels, roots are a prime source of carbon in soil. This makes roots preferential targets for soil-dwelling herbivores such as insects, nematodes, and other microbes. However, roots possess defense mechanisms that allow them to resist herbivore attacks (see Erb et al., 2012, this issue). Indeed, they have evolved a broad arsenal of direct defense molecules as well as indirect defenses that involve finely tuned communication and chemical interactions of the roots with the soil

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microfauna (Huber-Sannwald et al., 1997; Boff et al., 2001; van Tol et al., 2001; Mathesius et al., 2003; Callaway et al., 2004; Rasmann et al., 2005, 2011; Ali et al., 2010). In this review, we highlight some of these chemically mediated interactions (Fig. 1), and we argue that the chemical cues that are involved can be used to improve belowground pest control and crop production.

Plant–Insect Interactions and Belowground Pest Management

Rasmann and Agrawal (2008) estimate that about 17 % of all insect families of North America contain species of root feeders (including chewers, sap suckers, and gall makers). Common insect orders such as Orthoptera, Lepidoptera, Diptera, Homoptera, Coleoptera, and Hymenoptera have immature root-feeding instars (Brown and Gange, 1990). Because of their direct impact on plant development and fitness, root-feeding insects play an important role in both agricultural and natural ecosystems (Blossey and Hunt-Joshi, 2003; Wardle et al., 2004; Rasmann and Agrawal, 2008). Indeed, as belowground herbivory induces changes in the physiology and morphology of the roots, soil-dwelling herbivores have the potential to shape the ecosystems at the plant community level (De Deyn et al., 2003), belowground fauna (Wardle, 2006), as well as aboveground insect communities (Bezemer and van Dam, 2005).

Various volatile organic compounds have been identified as arthropod attractants belowground. A comprehensive review by Wenke et al. (2010) provides an inventory of a wide range of compounds used by belowground insect herbivores to locate their food source. Johnson and Nielsen (2012, this issue) discuss in detail how insect–plant interactions are mediated by belowground volatiles. The simplest and most ubiquitous of such signals in the soil is carbon dioxide (CO_2) emitted by respiring roots, but also many other biotic sources. Johnson and Gregory (2006) listed more than 20 studies in which CO_2 was shown to be a major attractant for root feeding arthropods. Whereas low concentrations are known to trigger chemotaxis and attract insects, high concentrations of CO_2 may actually result in disorientation (Johnson and Gregory, 2006). CO_2 is such an ambiguous signal that is unlikely to be of great use by itself. We, therefore, recently argued that CO_2 is a response activator rather than a key attractant *per se* (Turlings et al., 2012). This notion is based on the principle that where there are roots there is CO_2, whereas the reverse does not hold; where there is CO_2 there are not necessarily roots. The same idea holds for hemophagous insects in search of a blood meal

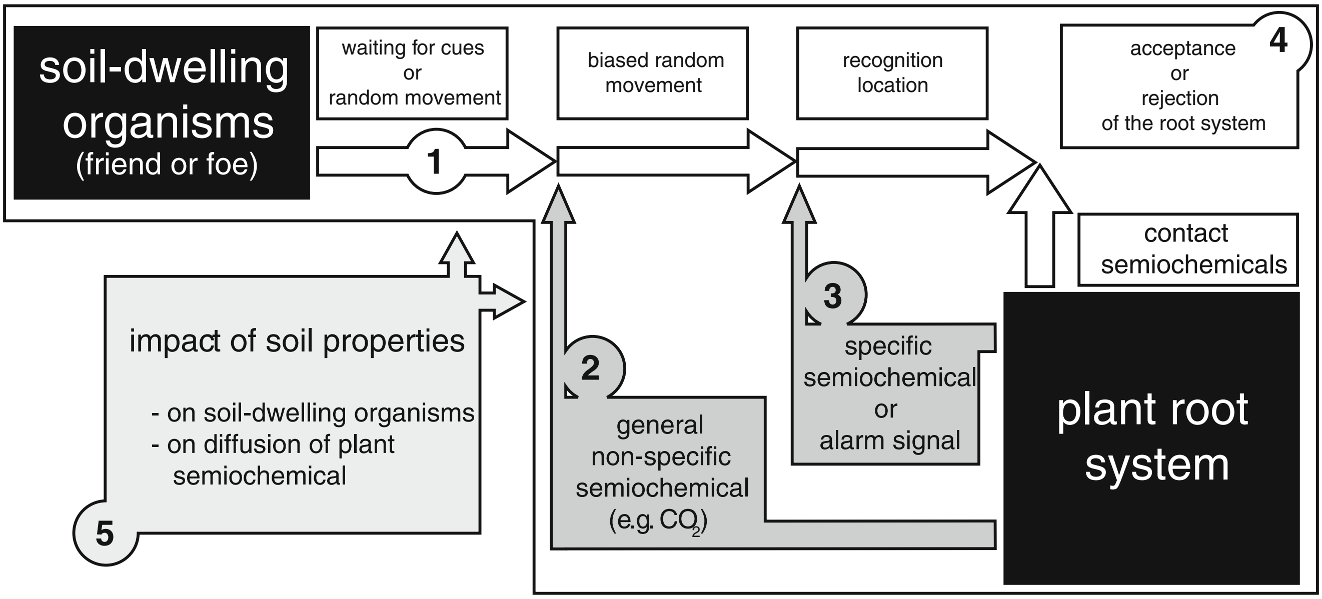

Fig. 1 Schematic representation of chemically-mediated interactions between plants and soil-dwelling organisms. (*1*) Without root signal, the organisms are either waiting for cues or randomly move around until they detect a chemical cue. (*2*) General nonspecific semiochemicals emitted by roots may trigger a shift from random movement or immobility to a biased random movement. (*3*) More specific chemical cues may allow the organism (friend or foe) to recognize and locate a potential partner to establish an interaction with. (*4*) Subsequent acceptance or rejection takes place at the surface of roots due to the presence of contact chemosensory cues, being either feeding stimulants or deterrents for herbivorous organisms or cues that indicate a partner for the establishment of a mutually beneficial interaction. (*5*) Soil properties have an obvious impact on soil chemically mediated interactions; the clay-humic complex may favor or slow down the diffusion of the volatiles depending on the chemical interactions taking places at this interface. Moreover, soil porosity, connectivity, or particle size distribution impact the mobility and behavior of soil-dwelling organisms. Understanding each of these steps will allow us to manipulate the system in order to favor or to inhibit beneficial and detrimental interactions, respectively [modified after Johnson and Gregory (2006)]

and indeed it has been found that the presence of CO_2 strongly increases their responsiveness to more specific cues (Dekker et al., 2005; Turner et al., 2011). Indeed, besides CO_2, there are several compounds that have been identified as potent specific attractants to root feeders. For instance, several disulfides and trisulfides attract root-feeding larvae of the fly *Delia antiqua* in *Allium cepa* (Carson and Wong, 1961). Fatty acids in oaks (*Quercus* sp.) and monoterpenes in carrots (*Daucus carota* ssp. *sativus*) attract larvae of the forest cockchafer, *Melolontha hippocastani* (Weissteiner and Schütz, 2006). Johnson et al. (2005) showed the attraction of *Sitona lepidus* to formononetin, a flavonoid emitted by nodualted roots of white clover *Trifolium repens.* In laboratory assays, the scolyt beetle *Hylastinus obscurus* was shown to be attracted to volatile exuded by roots of red clover *Trifolium pretense* (Quiroz et al., 2005).

Johnson and Gregory (2006) proposed a conceptual model for chemically mediated plant host location and acceptance by belowground insect pests that can readily be adapted to general belowground chemical signaling pathways (Fig. 1). Interfering with any of these steps would disrupt the insect's ability to find or accept its host, and thus offers a way to control pest insects. Following this approach, Bernklau et al. (2004) managed to interfere with the host-finding behavior of the larvae of the western corn rootworm *Diabrotica virgifera virgifera*. The larvae of this chrysomelid beetle are an important pest of maize, and rely, among other volatile cues, on CO_2 to locate the root system of its host (Bernklau and Bjostad, 1998a, b). In a laboratory assay, significantly fewer *D. v. virgifera* larvae were recovered from maize roots in soil with CO_2-producing granules than from maize roots in control soil, suggesting that the increase in CO_2 prevented the insect larvae from locating the roots of their host plant (Bernklau et al., 2004). By testing the same strategy in the field, they found that CO_2 application resulted in a significant decrease in damage done by *D. v. virgifera* to the maize root (Bernklau et al., 2004).

In an earlier study, Bjostad and Hibbard (1992) identified a more specific cue, the 6-methoxy-2-benzoxazolinone (MBOA), as an attractant for *D. v. virgifera* larvae. MBOA is one of several benzoxazinoids that maize seedlings produce and release as toxic and anti-feedents against insects in soil (Bjostad and Hibbard, 1992). *D. v. virgifera* have evolved resistance to benzoxazinoids (Abou Fakhr et al., 1994; Robert et al., 2012a) and even rely on this cue to locate the host plant (Bjostad and Hibbard, 1992) and to identify the most nutritious maize roots (Robert et al., 2012a). Knowing the importance of MBOA as a foraging cue for the pest, Hibbard et al. (1995) employed it to reduce larval damage on maize roots in the field. They baited a soil insecticide with MBOA to lure *D. v. virgifera* larvae to their death (Hibbard et al., 1995). Similarly, Bernklau and Bjostad (2005) could reduce the effective dose of the insecticide thiamethoxan by 50 % when they mixed it with feeding stimulants. Recently, attempts to lure foraging *D. v. virgifera* larvae by using alginate capsules as dispensers of attractants and feeding stimulants have been undertaken. In the laboratory, larvae of the chrysomelid pest were found to be as much attracted towards the capsules as towards the roots of a maize seedling. However, in the field, the attractive coating of the capsules did not help to further reduce *D. v. virgifera* damage on the maize roots (Hiltpold et al., 2012). Hence, this approach needs to be improved, but it has interesting potential in pest management, especially because the capsules can be used to deliver biocontrol agents such as entomopathogenic nematodes (Hiltpold et al., 2012) into pest-infested fields. By luring the pests towards the capsules, their efficacy can be further enhanced.

Even though examples are scarce, it is evident that the manipulation of chemically mediated host recognition and/or food acceptance has great potential in controlling insect pests (Fig. 1). However, basic knowledge on chemical attraction of pests towards their host and the chemical cues that they use as host acceptance signals is largely missing. Having such information would help breeders to select varieties with the right chemical profile, or it might even be possible to genetically engineer plants to make them emit less attractive volatiles or even repel the pests. Thus, affecting the acceptance of food sources by an insect herbivore could provide ecologically sound solutions to pest problems.

Belowground Tritrophic Interactions as an Inspiration for Insect Pest Control Strategies Plants cannot run away to escape herbivory, but they have evolved many other defense traits (Howe and Jander, 2008). One strategy that appears to provide protection against herbivory is the release of herbivore induced volatile organic compounds (Fig. 1), which increases the plant's attractiveness to the natural enemies of herbivores (e.g., Dicke and Vet, 1999; Dicke et al., 2003; Turlings and Wäckers, 2004; Kessler and Morrell, 2010). Such interactions also take place belowground. For instance, females of the predatory mites *Neoseiulus cucumeris* respond to belowground volatiles signals of tulip bulbs infested by the rust mite *Aceria tulipae*, but not to volatiles of untreated or mechanically wounded bulbs (Aratchige et al., 2004). Single root-emitted chemicals can have a dual beneficial effect for the plant. For instance, dimethyl disulfide is emitted from cabbage roots damaged by the cabbage root fly *Delia radicum* (Ferry et al., 2007; Danner et al., 2012, this issue). This volatile both attracts the main predators of *D. radicum* (i.e., two staphylinids, *Aleochara bilineata* and *Aleochara bipustulata*, and carabid beetles of the genus *Bembidion*) and it inhibits oviposition by cabbage root fly females (Ferry et al., 2009). In a field experiment, the authors placed dispensers to continuously release dimethyl disulfide in broccoli plots. The number of predators increased in the plots that received the dispensers (Ferry et al., 2009). In this particular experiment, the increase in predators did not improve the quality of the harvested plants at the end of the season, but such approaches should help pest management at higher pest densities.

Boff et al. (2001) and van Tol et al. (2001) found that the emission of odorous volatiles by insect damaged roots results in the attraction of entomopahtogenic nematodes. These insect-killing microscopic worms are frequently used in insect–pest management (Grewal et al., 2005), but rarely in large-scale agriculture. Exploiting their ability to detect damaged roots might be extremely interesting in the context of pest control improvement. To date, only few additional tritrophic interaction that rely on belowground herbivore-induced volatiles have been described in agricultural ecosystems (Rasmann et al., 2005; Rasmann and Turlings, 2008; Ali et al., 2010) or in natural ecosystems (Rasmann et al., 2011). Ali et al. (2010) recently showed that the entomopathogenic nematode *Steinernema diaprepesi* is significantly more attracted by citrus roots damaged by the larvae of the curculionid pest *Diaprepes abbreviates* than by mechanically damaged roots. However, this agronomically interesting trait also is abused by pests, as insect-induced roots of citrus tree also attract the plant parasitic nematode *Tylenchulus semipenetrans* (Ali et al., 2011). Consequently, this may interfere with the possible exploitation of citrus induced volatiles in biological control strategies that target *Diaprepes abbreviates*, specifically in cases where rootstocks are not naturally resistant to this nematode pest.

One of the best studied belowground tritrophic interactions involves maize roots (Rasmann et al., 2005). Upon attack by the voracious larvae of *D. v. virgifera*, the roots of many maize varieties emit the sesquiterpene (*E*)-β-caryophyllene (Rasmann et al., 2005; Köllner et al., 2008), which is highly attractive to the entomopathogenic nematode *Heterorhabditis megidis* in the laboratory as well as in the field (Rasmann et al., 2005; Köllner et al., 2008; Hiltpold et al., 2010c). However, most of the American maize varieties have lost the ability to produce (*E*)-β-caryophyllene (Rasmann et al., 2005; Köllner et al., 2008), probably because the herbivore induced cue also recruits *D. v. virgifera* larvae (Robert et al., 2012b), which may have changed breeders to unintentionally select against this trait. Nevertheless, plants that do not emit this signal may suffer from more rootworm damage than plants that are able to recruit the entomopahtogenic nematodes (Rasmann et al., 2005; Hiltpold et al., 2010c, 2011). To restore the ability of maize to indirectly protect its roots with the emission of (*E*)-β-caryophyllene, the terpene synthase gene *Ovtps6* from *Oreganum vulgare* (Crocoll et al., 2010) was introduced to a maize variety that normally is unable to produce the sesquiterpene (Degenhardt et al., 2009). The transformation resulted in maize lines that constitutively emitted (*E*)-β-caryophyllene (Degenhardt et al., 2009). When these transformed lines were compared to untransformed isogenic lines, significantly more nematodes *H. megidis* were attracted toward the genetically engineered plants than toward the controls both in the laboratory and in the field, resulting in a better protection of the emitting roots (Degenhardt et al., 2009). This first field demonstration that genetic engineering can be used to enhance indirect defenses against insect pests illustrates the potential of exploiting plant mediated signaling for crop protection. However, such approach is feasible only in combination with the right species of nematode (Hiltpold et al., 2010c). In fact, *Heterorhabditis bacteriophora*, which is highly virulent against *D. v. virgifera* (Kurzt et al., 2009), does not respond well to (*E*)-β-caryophyllene (Hiltpold et al., 2010c). To overcome this drawback, a strain of *H. bacteriophora* was selected in the laboratory for enhanced responsiveness to (*E*)-β-caryophyllene (Hiltpold et al., 2010a). The selection resulted in a strain that responded 6-fold better than the original strain and with equivalent virulence and persistence (Hiltpold et al., 2010a, b). The application of this strain in the field significantly increased the mortality of *D. v. virgifera* larvae feeding on the roots of plants emitting (*E*)-β-caryophyllene (Hiltpold et al., 2010a). A recent study on chemotaxis of *H. bacteriophora* and *Steinernema feltia* has revealed several new compounds that induce movement in the tested entomopahtogenic nematodes (Hallem et al., 2011). Further research is needed to determine the full potential of using these belowground signals for insect pest control.

Only few inducible and constitutively emitted volatiles involved in belowground tritrophic interactions are known, but an increasing effort is invested in this field of research. Little is known also about the impact of abiotic factors in the soil on the diffusion of these volatiles (Hiltpold and Turlings, 2008) or about the foraging behavior of the beneficials such as the nematodes (Kruitbos et al., 2010; Wilson et al., 2012). Understanding more about the complex interactions at each trophic level will not only reveal the intricacies of these fascinating interactions in the rhizosphere, but may also lead to ecologically sound alternatives in pest management in agricultural systems.

Management of Plant Parasitic Nematodes Using Root-Produced Exudates

After insects, the second most important group of root feeders encompasses the plant parasitic nematodes. All species are obligate parasites, feeding exclusively on the cytoplasm of living plant cells. The most economically important groups of nematodes are the sedentary endoparasites including the genera *Heterodera* and *Globodera* (cyst nematodes) and *Meloidogyne* (root-knot nematodes). Cyst and root-knot nematodes differ in their parasitic life-cycle strategies, but they both rely on volatile cues to locate the host plant. With the exception of ambiguous CO_2 emissions, it is largely unknown what triggers the attraction of plant parasitic nematode towards host plants (discussed by Rasmann et al., 2012, this issue). Carbon dioxide has been shown to attract several nematode species (Klingler, 1963; Dusenbery, 1980, 1987; Pline and Dusenbery, 1987), but aggregation and attraction of plant parasitic nematodes also have been demonstrated in response to plant root exudates (Prot, 1980; Rolfe et al., 2000; Curtis et al., 2009; Reynolds et al., 2011). Only recently, has it been found that plant parasitic nematodes follow gradients of herbivore-induced terpene volatile organic compounds (Ali et al., 2011). In their study, a series of terpenoids were identified as possible attractants for the nematode *Tylenchulus semipenetrans*, including α- and, β-pinene, limonene, geijerene, and pregeijerene (Ali et al., 2011). The identification of such specific volatiles offers the possibility of employing a confusion strategy to disrupt nematodes' host location and acceptance efforts, analogous to the pheromone confusion technique used in insect pest control (e.g., Joshi et al., 2011; Levi-Zada et al., 2011; Vacas et al., 2011, 2012; Schmera and Guerin, 2012). Further research will be needed to understand fully the mechanisms behind nematode attraction in order to develop lures that can compete with the plant-produced attractants.

On the one hand, root exudates may attract plant parasitic nematodes, but they are also involved in plant defense against these pests. For instance, root tip exudates can trigger a loss of motility, inducing quiescence and thus reducing the ability of the nematodes to successfully infect the plant (Zhao et al., 2000). Such temporal alteration of plant parasitic nematode motility in contact with root exudates has been observed for several plant species (Hubbard et al., 2005), suggesting that this defense strategy is widespread. Attempts to identify the active compounds have so far failed (Hubbard et al., 2005). Once identified, synthetic versions of the active compound(s) might be employed by spraying them to immobilize plant parasitic nematodes in the field. It is evident that further fundamental research into possible other ecological roles of such compounds is essential in order to establish whether or not they could be ecologically sound alternatives in plant parasitic nematode control.

Because plant parasitic nematodes rely on plants as food sources, they not only use plant chemicals to locate roots, but they also synchronize egg hatching with the phenology of their host plants. It has been amply demonstrated that plant parasitic nematode egg hatching is stimulated by root exudates (e.g., Perry and Clarke, 1981; Perry and Gaur, 1996; Dennijs and Lock, 1992; Gaur et al., 2000; Devine and Jones, 2001; Wesemael et al., 2006; Pudasaini et al., 2008; Khokon et al., 2009; Oka and Mizukubo, 2009). For instance, a key hatch-stimulating substance for soybean cyst nematode was successfully isolated from soybean roots (Masamune et al., 1982). Sometime later, solanoeclepin A, a hatching stimulus for the potato cyst nematodes *Globodera rostochiensis* and *G. pallida* Stone, was isolated by Mulder et al. (1992) and its structure was resolved by Schenk et al. (1999). It is easy to imagine various applications of such compounds in crop protection; these compounds can be applied to the field before the plants have been sown or germinated. This should result in nematode hatching in the absence of their actual host plants, and the free-roaming nematode can be expected to die of starvation or chemical pesticides before damages occur. However, the challenge of this idealistic plan of attack is the availability of enough material to treat large crop fields. Until recently, the hatch-stimulating chemicals have been isolated only in minute quantities from natural sources, but Tanino et al. (2011) have developed a potent laboratory synthesis methodology of solanoeclepin, thus opening the way to a new management strategy of plant pathogenic nematodes.

Specific root secondary metabolites or breakdown products also have a direct impact on plant parasitic nematode survival. For instance, Brassicaceae plants contain various glucosinolates (McCully et al., 2008) that are released upon pest damage and degraded into toxic breakdown products such as (iso)thiocyanates (Halkier and Gershenzon, 2006). Belowground, glucosinolates and their breakdown products can efficiently reduce the populations of plant parasitic nematodes (e.g., Potter et al., 1998, 2000; Lazzeri et al., 2004; Oliveira et al., 2011). The release of these toxic compounds into the soil does not alter communities of beneficial organisms such as earthworms or collembola (Kabouw et al., 2010), and this approach has been widely used for the protection of subsequent crops (Matthiessen and Kirkegaard, 2006; Lazzeri et al., 2010). Moreover, breeding for increased concentrations of glucosynolates in roots of *Brassica* plants has resulted in a better control of nematode pests (Potter et al., 2000), and can be an effective way to manipulate belowground chemical ecology to control plant parasitic nematodes.

Enhancing Plant Production by Exploiting Chemically Mediated Interactions Between Roots and Microbes

Plants have to face several foes in soil, but they also can interact with beneficial microbes to increase their biomasses or, in agriculture, yield. Indeed, there are myriads of microorganisms that interact with plants with different levels of intimacy, ranging from symbionts to co-inhabitants of the same niche without particular interaction, and each interaction might be of interest in the context of plant protection and production.

Plant Interactions with Free-Living Nematodes There are numerous free-living nematodes in soil that do not need an insect or a host plant to complete their life cycle. These nematodes are usually bacterivorous, carnivorous, or fungivorous (Neher, 2010). Nonetheless, they can interact with plant roots in various negative or positive ways. On the negative side, they transmit viruses or plant pathogenic bacteria (Raaijmakers et al., 2009). On the positive side, they also can carry beneficial microorganisms and enhance root growth. For instance, *Caenorhabditis elegans* mediates positive interactions between plant roots and rizhobia, thus resulting in a increased number of bacterial colonies (Horiuchi et al., 2005) and potential increases in nodulation. *Caenorhabditis elegans* are attracted by dimethyl sulfide toward *Medicago truncatula*, and thereby transport the beneficial rizho-bacterium *Sinorhizobium meliloti* close to the root systems (Horiuchi et al., 2005). Nematodes also are able to carry fungal spores that adhere to their cuticular mucilage (Bonkowski et al., 2009), and thus they serve as potential vectors for beneficial plant symbiotic fungi. Beside these transporter activities in the rhizosphere, free-living nematodes also enhance plant nutrient availability by grazing on microbial communities and increasing their turnover and metabolic activity (Bonkowski et al., 2009). Obviously, nematodes are not the only animal feeding on bacteria in the rhizosphere. Other organisms such as amoeba also positively impact nutrient turnover around roots (Rosenberg et al., 2009). A better understanding of such interactions and knowledge of the chemicals that are involved in their establishment could lead to novel strategies to enhance nutrient availability and uptake in the rhizosphere. The favoring of natural nutrient cycles in crop production also will reduce the need for fertilizer input and can contribute to a more sustainable agriculture and food production.

Root Volatile Involved in Communication with Symbiotic Fungi Simple root volatile organic compounds such as carbon dioxide play an important generic role in belowground interactions with other organisms (Johnson and Gregory, 2006). However, CO_2 also has been shown to mediate highly specific interactions. Indeed, carbon dioxide is crucial in the growth of the vesicular-arbuscular fungus *Gigaspors margarita*, an obligate biotrophic symbiont (Bécard and Piché, 1989). A synergistic effect of CO_2 and root exudate factors in the hyphal growth was measured; carbon dioxide and root exudates taken alone had little or no effect, but when mixed together, they significantly stimulated hyphal growth (Bécard and Piché, 1989). Further experimentation has suggested that, in this particular interaction, carbon dioxide serves as an essential source of carbon for fungal growth (Bécard and Piché, 1989). Since then, numerous plant exudates, mainly belonging to the sesquiterpene lactone family, have been shown to mediate plant–microbe interactions. For example, the strigolactone 5-desoxy-strigol, isolated from *Lotus japonicus*, triggers hyphal branching in *G. margarita* (Akiyama et al., 2005). Very recently, the first component involved in strigolactone root exudation has been described (Kretzschmar et al., 2012). The identification of the ABC transporter in *Petunia ssp.* opens new opportunities to manipulate strigolactone dependent processes (Badri et al., 2009; Kretzschmar et al., 2012).

Conclusion

This review summarizes our current knowledge of direct and indirect interactions between soil fauna, rhizosphere microorganisms, and plant roots, and highlights the importance of such knowledge for the development of methods to fight soil pests. Research into belowground chemically mediated interactions is drastically increasing, and no longer is restricted to interactions between roots and microbial symbionts, but involves many other soil-dwelling organisms. It is increasingly recognized that, similar to aboveground interactions, a coevolution between plants and herbivores has taken place belowground that has led to sophisticated reciprocal adaptations. Microbial communities (see Effmert et al. 2012, this issue) and bacterivorous fauna jointly have strong effects on root growth and architecture, even though plants might only be passive benefiters and not directly shape these interactions. Conversely, an increasing number of examples suggest that roots are active players in the rhizosphere and that they are able to influence and shape their environment, thus ensuring their protection and optimizing their performance. They have been shown to chemically influence soil microorganisms and fauna for their own benefit: entomopahtogenic nematodes are recruited by insect herbivore damaged roots; plant pathogenic nematodes are immobilized by root tip exudates; and root diffusates can attract free-living nematodes that carry potentially beneficial bacteria and initiate symbiosis between plants and beneficial fungi.

In our efforts to exploit root signals in crop protection, it should be realized that herbivorous insects also may use root signaling to locate their food source, and that root chemicals can trigger egg hatching in plant parasitic nematodes. Only a multidisciplinary approach to disentangle all aspects of root ecology will allow us to use chemically mediated belowground interactions to our benefit. Special effort should be invested in understanding the role of fungi in belowground interactions, indeed, as “root extensions”, hyphae must play a central role in local as well as in long distance belowground signaling. Co-evolutionary perspectives are lacking in rhizosphere ecology and belowground food webs. A good understanding of these processes would help in approaches that conserve well-established beneficial interactions during domestication and breeding of cultivars. In general, analytic methodologies that are employed for the description of aboveground interactions are in part transferable to belowground chemical ecology. It is important to note that, before the techniques that are discussed here can be applied, it is essential to evaluate the overall consequences of the manipulations. Hundreds of species of microorganisms can be found in a handful of soil and changing one parameter might have unexpected consequences on the established ecosystem services and threaten soil sustainability. Because soils are complex and heterogeneous ecosystems, the application of various strategies cannot be generalized, and will have to be carefully assessed in case by case studies. Hence, with the increasing interest in what might be called a new frontier in biological sciences, a cooperative and holistic approach appears crucial to tackle the complexity of the rhizosphere. This should allow us to benefit optimally from generated knowledge for sustainable agricultural practices.

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REVIEW ARTICLE

Mycorrhiza-Induced Resistance and Priming of Plant Defenses

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Abstract Symbioses between plants and beneficial soil microorganisms like arbuscular-mycorrhizal fungi (AMF) are known to promote plant growth and help plants to cope with biotic and abiotic stresses. Profound physiological changes take place in the host plant upon root colonization by AMF affecting the interactions with a wide range of organisms below- and above-ground. Protective effects of the symbiosis against pathogens, pests, and parasitic plants have been described for many plant species, including agriculturally important crop varieties. Besides mechanisms such as improved plant nutrition and competition, experimental evidence supports a major role of plant defenses in the observed protection. During mycorrhiza establishment, modulation of plant defense responses occurs thus achieving a functional symbiosis. As a consequence of this modulation, a mild, but effective activation of the plant immune responses seems to occur, not only locally but also systemically. This activation leads to a primed state of the plant that allows a more efficient activation of defense mechanisms in response to attack by potential enemies. Here, we give an overview of the impact on interactions between mycorrhizal plants and pathogens, herbivores, and parasitic plants, and we summarize the current knowledge of the underlying mechanisms. We focus on the priming of jasmonate-regulated plant defense mechanisms that play a central role in the induction of resistance by arbuscular mycorrhizas.

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Introduction

Arbuscular mycorrhizas are mutualistic associations formed between the roots of 80 % of terrestrial plant species and fungi from the small phylum *Glomeromycota* (reviewed by Schüßler et al., 2001). The symbiosis is named after the Greek “*mycos*” and “*rhiza*” meaning “fungus-root,” and it is probably the oldest and most widespread plant symbiosis on Earth. Indeed, fossil records and phylogenetic evidence date their existence back more than 450 million years (Smith and Read 2008), which indicates a considerable selective advantage for both partners. Arbuscular mycorrhiza-forming fungi (AMF) are obligate biotrophs that require the host plant to complete their life cycle. The fungus colonizes the root cortex and forms intracellular structures called arbuscules (from the Latin “*arbusculum*”, meaning bush or little tree) where the exchange of nutrients between the partners takes place. The extracellular hyphal network spreads widely into the surrounding soil, thereby reaching out of the nutrient depletion zone and improving the supply of inorganic nutrients, especially phosphate and nitrate (Smith et al., 2011). In return, the heterotrophic fungal partner receives photosynthates from the host plant (Smith and Smith, 2011). Mutual benefits are the basis of the evolutionary success of the interaction, ensured through a tight bidirectional control of the mutualism (Kiers et al., 2011). From the plant side, this regulation implies important changes in the plant primary and secondary metabolism and regulation of the plant defense mechanisms (Harrison, 1999; Hause and Fester, 2005). These changes usually have a deep impact on plant physiology, altering the plant’s ability to cope with stresses.

Early studies on mycorrhizas showed an improved growth and/or yield of mycorrhizal plants, first attributed exclusively to the improved nutritional status of the plant (reviewed in Linderman, 1994). Later, several authors reported a higher tolerance of mycorrhizal plants to abiotic stresses, such as drought, salinity, or presence of heavy metals (Miransari, 2010; Smith et al., 2010). Evidence also has accumulated on the higher resistance of mycorrhizal plants to a wide range of below-ground attackers such as soil-borne fungal and bacterial pathogens, nematodes, or root-chewing insects (Azcón-Aguilar and Barea, 1997; Whipps, 2004). Only in the last decade, has induced resistance against shoot pathogens also been reported (Pozo and Azcón-Aguilar, 2007; Koricheva et al., 2009; Campos-Soriano et al., 2012, Jung et al. unpublished). The need for assistance in overcoming stressful conditions has been considered one of the explanations for mycorrhizas' persistence during evolution, even in systems where the symbiosis does not confer growth benefits (Newsham et al., 1995). With regard to its biofertilizer and bioprotective properties, the mycorrhizal symbiosis has become a focal point of research as an alternative to chemical fertilizers and pesticides in sustainable agriculture (Harrier and Watson, 2004; Mukerji and Ciancio, 2007; Fester and Sawers, 2011).

Despite the obvious benefits of an improved nutritional status for stress tolerance/ resistance, mineral supply experiments have shown that the protective effect observed in mycorrhizal plants cannot be attributed to improved nutritional status alone (Fritz et al., 2006; Liu et al., 2007). AM associations bring about significant changes in the host plant and its environment: at the rhizosphere level, they influence soil structure, carbon deposition in soil, and microbial diversity, in part through changes in root exudation. These shifts in the microbial communities of the rhizosphere may indirectly influence the out-come of plant interactions with other organisms, including pathogens and beneficial microbes (Berta et al., 2002; Barea et al., 2005; Artursson et al., 2006; Lendzemo et al., 2007; see also Cipollini et al., 2012, this issue; Effmert et al., 2012, this issue). Apart from the changes in the rhizosphere, multiple modifications also occur within the host plant. In the roots, changes in architecture, alterations of the metabolic profile, and accumulation of certain defense compounds may occur (García-Garrido and Ocampo, 2002; Strack et al., 2003; Hause et al., 2007; Schliemann et al., 2008; Péret et al., 2009; López-Ráez et al., 2010a, b). For example, the accumulation of apocarotenoids (cyclohexenone and mycorradicin derivatives) can be observed in mycorrhizal roots, which are the main component of the yellow pigment found in many plant species upon colonization by AMF and have been proposed to play a role in control of the degree of colonization and mycorrhizal functionality (Strack et al., 2003; Strack and Fester, 2006; Floß et al., 2008; Schliemann et al., 2008). Qualitative and quantitative changes in flavonoid contents have been observed, the changes depending on the host plant, AMF, and developmental stage of the symbiosis (Vierheilig and Piché, 2002; Akiyama et al., 2002). Changes in phenolic compounds, defense-related phytohormones, and reactive oxygen species also have been reported (Fester and Hause, 2005; López-Ráez et al., 2010a; b). Noteworthy, the symbiosis also has a considerable impact on the aerial parts of mycorrhizal plants, some of the reported changes being related to defense or stress tolerance (Liu et al., 2007; Kaschuk et al., 2009; Fiorilli et al., 2009; Pozo et al., 2009; Fester et al. 2011; Aloui et al., 2011).

As for the higher resistance to pests and pathogens of AMF-colonized plants, observations of systemic protection against pathogens in non-colonized root fragments from mycorrhizal plants and enhanced resistance of the aerial parts to certain attackers have pointed out the involvement of plant defense mechanisms (Cordier et al., 1998; Pozo et al., 2002; Pozo and Azcón-Aguilar, 2007). Defense mechanisms are coordinated by the plant immune system, strikingly similar in some aspects to the innate immune system in animals (Ausubel, 2005). This system allows the plant to distinguish non-self alien organisms by recognizing structurally conserved microbe-associated molecules, such as flagellin, lipopolysaccharides, or peptidoglycans, which are collectively termed microbe-associated molecular patterns (MAMPs, or PAMPs in the case of pathogens). PAMPs are recognized by transmembrane pattern recognition receptors (PRRs), which leads to the induction of the appropriate responses in the host and to PAMP-triggered immunity (PTI) (Ausubel, 2005; Jones and Dangl, 2006; Boller and He, 2009; Thomma et al., 2011). In an evolutionary "arms race," microbes have evolved effector proteins that are secreted into the host and suppress PTI, thus allowing successful host colonization by the pathogen, thus causing effector-triggered susceptibility of the plant to the disease. In some cases, intracellular proteins of the plant recognize pathogen effectors or their modified target proteins and activate immune responses that are quicker, more prolonged, and more robust than those in PTI, resulting in effector-triggered immunity (ETI) (Jones and Dangl, 2006; Boller and He, 2009; Thomma et al., 2011).

Plant defense responses are coordinated by small molecules that act as signal transducers and tailor the coordinated expression of genes that code for defense-related proteins and compounds (Ausubel, 2005; Jones and Dangl, 2006). Among these molecules, the phytohormones jasmonic acid (JA), salicylic acid (SA), abscisic acid (ABA), and ethylene (ET) play key roles (Pieterse et al., 2009). According to the challenger lifestyle, one signaling pathway will prevail over the others. It is generally assumed that the SA-dependent pathway regulates responses such as programmed cell death, effective against biotrophic organisms, and the JA-dependent pathway regulates responses to necrotrophs and chewing insects (Glazebrook 2005). However, these hormone signaling pathways do not act independently, but influence each other through a complex network of regulatory interactions, JA and SA pathways in general being mutually antagonistic (Pieterse et al., 2008). As biotrophs, mycorrhizal fungi share some similarities with biotrophic pathogens, and are able to trigger plant defense responses at initial stages (Paszkowski, 2006). Thus, for a successful colonization, the fungus has to cope with these reactions and actively modulate plant responses. We have proposed that this modulation may result in pre-conditioning of the tissues for efficient activation of plant defenses upon a challenger attack, a phenomenon that is called *priming* (Pozo and Azcón-Aguilar, 2007).

Priming sets the plant in an "alert" state in which defenses are not actively expressed but in which the response to an attack occurs faster and/ or stronger compared to plants not previously exposed to the priming stimulus, efficiently increasing plant resistance. Thus, priming confers important fitness benefits (Conrath et al., 2006; Van Hulten et al., 2006; Walters and Heil, 2007). In the past decade, many priming-causing agents have been identified. It has been observed that some chemicals that induce stress responses in plants also induce priming when applied at lower doses, and several fungicides have been shown to prime defenses in treated plants in addition to their primary antifungal activity (reviewed in Conrath et al., 2006; Beckers and Conrath, 2007). Other well-studied examples of priming by chemicals include increased resistance to downy mildew in *Arabidopsis thaliana* after treatment with the non-protein amino acid β-aminobutyric acid (BABA), as well as primed defense responses in tomato and *Arabidopsis* pre-treated with hexanoic acid and subsequently infected with grey mold (Ton et al., 2005; Vicedo et al., 2009; Kravchuk et al., 2011). Remarkably, priming events occur as a result of inter-individual or even inter-species communication. For example, green leaf volatiles released by wounded or infested plants are also able to induce a more efficient activation of defenses in neighboring plants upon subsequent attacks (Kessler et al., 2006; Ton et al., 2007; Yi et al., 2009). In *Arabidopsis* seedlings exposed to volatile blends from two *Bacillus* species, the disease severity caused by a bacterial pathogen was significantly reduced (Ryu et al., 2004). Moreover, priming seems to be the mechanism underlying the Induced Systemic Resistance (ISR) observed in plants interacting with beneficial microorganisms (Conrath et al., 2006; Goellner and Conrath, 2008; Van Wees et al., 2008). Interestingly, priming of the plant immune responses by beneficial microbes is often dependent on a functional JA signaling pathway, as has been described for rhizobacteria and AMF (Verhagen et al., 2004; Pozo et al. 2004, 2010; van der Ent et al., 2009a). The molecular mechanisms behind priming of plant defenses and its biological relevance in plant resistance are now being uncovered (reviewed in Pastor et al., 2012), and evidence for trans-generational effects of priming have been a major advance in plant research (Luna et al., 2012; Rasmann et al., 2012; Slaughter et al., 2012). Here, we give a summary of the impact of the arbuscular mycorrhizal symbiosis on plant interactions with other organisms. We give special emphasis to the spectrum of protection against deleterious organisms (Mycorrhiza-Induced Resistance, MIR) and provide an overview of the underlying mechanisms, focusing on the priming of plant defenses associated with mycorrhization.

Mycorrhizal Associations Alters the Host Plant's Interactions with Other Organisms

As stated above, mycorrhization impacts plant interactions with other organisms. Due to potential practical applications, special attention has been devoted to the interaction with microbial pathogens or other deleterious organisms. In this section, we summarize the main effects of mycorrhization on biotic stress resistance (summarized in Fig. 1).

Mycorrhiza Effects on Below-Ground Interactions

Many studies show the protective effect of colonization by mycorrhizal fungi against infections by **microbial pathogens** in different plant systems. The majority of these reports focus on soil-borne pathogens such as fungi from the genera *Fusarium*, *Rhizoctonia*, *Macrophomina,* or *Verticillium*; bacteria such as *Erwinia carotovora*; or oomycetes like *Phytophthora*, *Pythium,* and *Aphanomyces*. In most cases, the protective effect is not only related to damage compensation or tolerance, but frequently the reduced damage also correlates with a decrease of the pathogen content within plant tissues (reviewed by Whipps, 2004). Similarly, there are many studies that show a clear reduction of the detrimental effects by endoparasitic **nematodes** such as *Pratylenchus* and *Meloidogyne* in mycorrhizal plants (Pinochet et al., 1996; De La Peña et al., 2006; Li et al., 2006; Elsen et al., 2008; Vos et al., 2011). Recently, a decrease on the development of ectoparasitic nematodes also has been described (Hao et al., 2012).

In contrast to the well-known effect on nematodes, there are relatively few studies on the impact of AMF on **root-feeding insects**, and they mostly focus on members of the genus *Otiorhynchus,* or weevils (Koricheva et al., 2009). The larvae of these insects are rhizophagous, whereas the adults feed on the foliage of the same plant. A clear protective effect of AMF is reported on the black vine weevil (*Otiorhynchus sulcatus*) that has a wide range of possible hosts (generalist). Experiments with strawberry showed that larval growth and survival was halved when the roots were

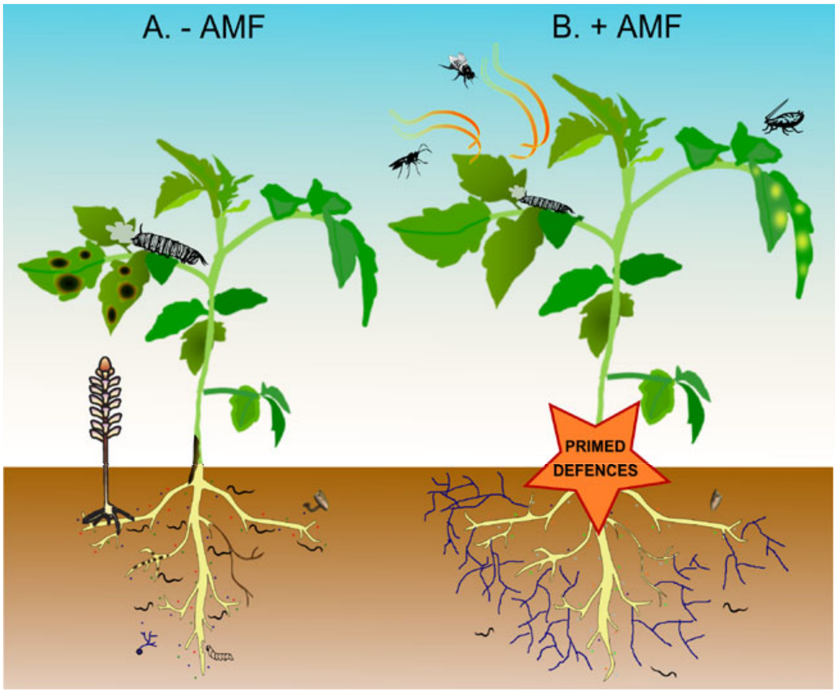

Fig. 1 **a Non-mycorrhizal plant (− AMF).** Absence of root colonization by AMF leads to stronger development of symptoms in response to necrotrophic pathogens and more damage upon feeding by chewing insects in roots and shoots when compared to mycorrhizal plants. Release of strigolactones (SLs) as part of the root exudates induces branching of AMF hyphae to promote mycorrhization but also induces germination of *Orobanchaceae* seeds which then parasitize the host plants‘ root system. **b Mycorrhizal plant (+AMF).** Growth promotion is often observed due to improved acquisition of mineral nutrients through the AM fungal hyphal network (represented in blue). Changes in the root exudate patterns repel nematodes and induce changes in the soil microbial community, possibly attracting antagonists of pathogens and a reduced release of SLs minimizes the risk of infection by root parasitic plants. Priming of plant defenses leads to a general reduction of the incidence and/ or damage caused by soil-borne pathogens, nematodes and chewing insects. In above-ground plant parts, viral and fungal biotrophs, as well as phloem-feeding insects, perform better on mycorrhizal plants. In contrast, the primed jasmonate-regulated plant defense mechanisms restrict the development of necrotrophic pathogens and the performance of phytophagous insects. Indirect defenses, such as the release of volatiles, are boosted and parasitoids are efficiently attracted

colonized by several *Glomus* species (Gange, 1996, 2001). In contrast, AMF colonization had no adverse effects on the growth of the specialist clover root weevil larvae (*Sitona lepidus*) that reached similar sizes independently of the mycorrhizal state of the host plant (Currie et al., 2011).

Beneficial organisms also are influenced by the presence of mycorrhiza. A positive effect of mycorrhization on **beneficial** plant-microbe interactions has been noted. For example, promotion of the interaction or synergistic effects have been described with regard to plant associations with nitrogen-fixing bacteria (Jia et al., 2004; Niranjan et al., 2007; Ferrari and Wall, 2008; Larimer et al., 2010); phosphate-solubilizing bacteria (Toro et al., 1996; Belimov et al., 1999; Kohler et al., 2007); biocontrol agents (Haggag and Abd-El Latif, 2001; Martínez-Medina et al., 2009; Martínez-Medina et al., 2010; Saldajeno and Hyakumachi, 2011); and plant growth–promoting microorganisms (Meyer and Linderman, 1986; Chandanie et al., 2005, 2006, 2009).

These effects on below-ground interactions may result from a combination of diverse mechanisms. Mycorrhizas compete for colonization sites with other microbes, and for example, full exclusion of the pathogenic oomycete *Phytophthora* from arbusculated cells has been described for tomato roots (Cordier et al., 1998). A competition for photosynthates also is possible between AMF and other microbes, especially with microbial symbionts, which require plant resources such as photosynthetically assimilated carbon. Colonization by AMF can lead to alterations in the quality and quantity of root exudates (Bansal and Mukerji, 1994; Azaizeh et al., 1995; Marschner et al., 1997; Sood, 2003; Pivato et al., 2008). These changes impact the microbial community of the mycorrhizosphere and, among other effects, may lead to a shift in its composition favoring certain components of the microbiota with the capacity to antagonize possible root pathogens (Barea et al., 2005; Badri and Vivanco, 2009). Altered root exudation also may directly impact microbial pathogens and nematodes. For example, exudates from mycorrhizal tomatoes transiently paralyzed nematodes, and generally their penetration into mycorrhizal tomato roots was decreased (Vos et al., 2011). Similarly, sporulation of the oomycete *Phytophthora fragrariae* was severely reduced in the presence of mycorrhizal strawberry root exudates (Norman and Hooker, 2000), and concentrated exudates from mycorrhizal tomato roots were repulsive to *Phytophthora nicotianae* zoospores (Lioussanne et al., 2008). Besides these repellent-like molecules, thus far, no antimicrobial compounds have been isolated from the exudates of mycorrhizal roots.

Altered root exudation also gives rise to the effect of mycorrhizas on plant interactions with **parasitic plants:** Mycorrhiza have been shown to reduce the incidence of root parasitic plants of the *Orobanchaceae*, including the genera *Striga*, *Orobanche,* and *Phelipanche* (López-Ráez et al., 2011b). These parasitic weeds are ubiquitous obligate parasites that cause immense yield losses in agriculture (Bouwmeester et al., 2003). It is known that seeds of these weeds germinate upon perception of strigolactones (SLs), a group of carotenoid-derived signaling molecules that are exuded by the roots of the host plant under conditions of phosphate starvation and promote AM hyphal branching and mycorrhiza establishment (Akiyama et al., 2005; Bouwmeester et al., 2007). The *Orobanchaceae* utilize this signal for the detection of an appropriate host plant that will be colonized and parasited. Interestingly, once the mycorrhizal symbiosis is well established, the SL production in the host plant goes down (Fig. 2; Lendzemo et al., 2007; López-Ráez et al., 2011a). This opens the possibility of using AMF in the control of parasitic weeds where conventional strategies have failed. Apart from their role as signaling molecules in the rhizosphere, SLs also play roles in signaling within the plant by acting in the regulation of shoot and root architecture. It is proposed that SLs, together with auxins, favor lateral root development that enables the root system to reach new areas in the soil where phosphate might be available (Stepanova and Alonso, 2009). The SL-mediated changes in root architecture may alter the dynamics of some pathogen infections, although direct evidence of such a correlation are lacking.

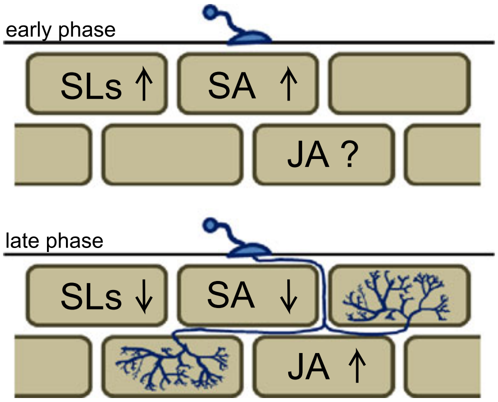

Fig. 2 Model for hormonal changes in the roots associated to the arbuscular mycorrhizal symbiosis. In the early phase of colonization strigolactone (SL) production is still high. Initially the AMF is perceived as an alien organism and as a consequence salicylic acid (SA) levels increased. In a well-established mycorrhiza both SL and SA production are repressed while biosynthesis of jasmonates (JA) increases

The use of experimental split-root systems has confirmed that the protection by mycorrhiza is manifested in non-colonized areas of the root system. For example, systemic protection in the root has been confirmed against oomycetes and bacterial pathogens in tomato (Cordier et al., 1998; Pozo et al., 2002; Zhu and Yao, 2004; Khaosaad et al., 2007), against fungal pathogens in barley (Khaosaad et al., 2007), and in banana and grapevine against nematodes (Hao et al., 2012). These experiments allow physical separation of the AMF and the aggressor, and they highlight the involvement of plant-mediated responses in the enhanced resistance, pointing out a major role for plant defense mechanisms.

Mycorrhiza Effects on Above-Ground Interactions

Systemic protection by a mycorrhizal association can even be observed in the aerial parts of a colonized plant, but in contrast to below-ground interactions, reports on AM effects on pests and pathogens attacking shoots are less studied, and the outcome of the interaction is more variable. Early studies described a higher susceptibility of AM plants to viruses, and biotrophic **pathogens** appear to thrive better on mycorrhizal plants, although an increased tolerance has been observed in terms of plant mass and yield (Gernns et al., 2001; Whipps, 2004). Concerning hemibiotrophs, the impact of the symbiosis varies from no effect to reduction of the disease, for example, against *Colletotrichum orbiculare* in cucumber (Lee et al., 2005; Chandanie et al., 2006). However, pathogens with a necrotrophic lifestyle are hampered in their proliferation, and symptom development is less severe on mycorrhizal plants. Examples are the fungi *Alternaria solani* in tomato (Fritz et al., 2006; de la Noval et al., 2007), *Magnaporthe grisea* in rice (Campos-Soriano et al., 2012), and *Botrytis cinerea* in roses and tomato (Møller et al., 2009; Pozo et al*.,* 2010).

In relation to **phytoplasmas**, specialized obligate parasites of phloem tissue that are transmitted by insect vectors, most reports show a reduction of disease incidence (Kamińska et al., 2010a, 2010b; Batlle et al., 2011; D'Amelio et al., 2011). However, it should be noted that the analysis of mycorrhizal effects on phytoplasmas and viruses is difficult because of the potential impact of mycorrhization on the insect vector and the complexity of studies on multi-trophic interactions.

Because of the multiple roles of insects in plant biology and their ecological relevance, the impact of mycorrhization on plant interactions with **insects** deserves special attention. Insects may be deleterious to plants by directly damaging them through herbivory or by acting as vectors for pathogens such as viruses and phytoplasmas, but they also can have positive effects on plant health by acting as natural enemies of pests or as pollinators. The outcome of the mycorrhiza-plant-herbivore interaction depends on many factors, such as the AM fungus, host and insect species involved, and environmental factors (Gange, 2007; Pineda et al., 2010). Several reviews have tried to compile the published studies dealing with these multitrophic interactions, most of them from an ecological point of view (Gehring and Bennett, 2009; Hartley and Gange, 2009). As a general pattern, the positive roles of insects on plants are favored by mycorrhization. For example, mycorrhization positively influence visitation rates of pollinating insects (Gange and Smith, 2005; Wolfe et al., 2005). This effect may be partly due to mycorrhizal effects on plant biomass, flower number and size, and amount of pollen produced (Gange and Smith, 2005). Similarly, the changes in the volatile profile of mycorrhizal plants under attack by herbivores result in a higher attraction of parasitoids that may help the plant to control the pest (Guerrieri et al., 2004; Leitner et al., 2010; Schausberger et al., 2011).

Concerning mycorrhizal effects on herbivorous insects, different outcomes have been reported. The improved growth and nutritional status of a mycorrhizal plant can compensate for feeding damage, whereas improved nutrient and water uptake facilitate the regrowth of tissues; all of which can increase host tolerance to herbivory (Kula et al., 2005; Bennett and Bever, 2007; Hoffmann et al., 2011).

Besides tolerance, direct effects on the insect also can occur. These effects largely vary depending on the type of the attacking insect. Generalist insects, able to feed on diverse plants and sensible to the plant defense mechanisms, are usually negatively affected by the presence of mycorrhizas (Rabin and Pacovsky, 1985; Gange and West, 1994; Vicari et al., 2002; Fontana et al., 2009). However, the outcome of the mycorrhiza-plant-herbivore interaction is not always favorable for the plant. Specialist insects, which feed from one or only a small number of hosts and show a high degree of adaptation to their hosts' defense responses, usually perform better on mycorrhizal plants, probably because of the improved nutritional quality of the host (Gehring and Bennett, 2009; Hartley and Gange, 2009). This general pattern, i.e., protection by mycorrhiza mostly against insect species sensitive to plant defense mechanisms, again supports the key role of the defense mechanisms on plant protection by mycorrhiza.

The degree of protection also depends on the feeding guild of the attacking herbivore. Phloem-sucking insects produce minimal damage to the plant while feeding and thereby avoid detection by the host's immune system (Walling, 2008). Thus, it is unlikely that potentiation of plant defense mechanisms in mycorrhizal plants may have a significant impact on them. Moreover, they may profit from its higher nutritional value. In fact, higher incidence of phloem-sucking insects in mycorrhizal plants has been reported (Gange et al., 1999; Goverde et al., 2000). In contrast, leaf chewers and miners usually are negatively affected by mycorrhization (Gange and West, 1994; Vicari et al., 2002). These insects feed on the leaf tissue and cause massive damage, which activates defenses that depend on the plant hormone JA (Howe and Jander 2008).

Taking into account the degree of specialization of the herbivore and its feeding guild, it can be summarized that mycorrhization negatively affects generalist leaf chewers, while having a positive or neutral effect on phloem feeders and specialist chewers (Gehring and Bennett, 2009; Hartley and Gange, 2009; Koricheva et al., 2009; Pineda et al., 2010). This spectrum of action strongly suggests that the effect of mycorrhization is related to priming of the plant defense mechanisms, and points out a prominent role of jasmonate signaling in the plant protection achieved by mycorrhization.

Mechanisms Underlying Mycorrhizas' Impact on Plant-Interactions with Pathogens and Pests

The effects of the AM symbiosis on plant interactions with other organisms, and, in particular, the induction of resistance against deleterious organisms seem to result from the combination of multiple mechanisms that may operate simultaneously. In the previous section, the possible role of competition for colonization sites and photoassimilates, and the contribution of the modifications in the microbial populations in the rhizosphere were discussed. Apart from that, a major transcriptional reprogramming takes place upon mycorrhizal colonization of the roots (Liu et al., 2003, 2007; Güimil et al., 2005; López-Ráez et al., 2010b). This reprogramming originates alterations in the primary and secondary metabolism in mycorrhizal plants (Hause et al., 2007; Toussaint, 2007; Schliemann et al., 2008). The majority of the changes affect the host's secondary metabolism, and have far-reaching consequences for the plant. One example is the alteration in root exudates, composed of various secondary metabolites such as phenolic compounds, strigolactones, and allelopathic compounds that regulate multiple interactions in the rhizosphere (Zeng, 2006; López-Ráez et al., 2010a, 2011b; Cipollini et al., 2012, this issue). Accordingly, all those changes may have special relevance to mycorrhizal effects on plant interactions below-ground. The symbiosis also increases the rate of photosynthesis and influences the carbon assimilation and allocation, thereby possibly affecting the source-sink relations that may influence the suitability of the plant for shoot attackers (Wright et al., 1998a, b). Obviously, improved nutrition in the plant also may account for damage compensation phenomena, and may, therefore, contribute to plant tolerance to diseases and herbivory. However, those mechanisms do not explain, for example, protection by AM under conditions where there are no nutritional benefits, and they do not explain the spectrum of action of mycorrhiza-induced resistance. These and other evidence discussed in the former section support the idea that the regulation of plant defenses during mycorrhization plays a major role in mycorrhiza induced resistance.

Modulation of the Host Plant's Immune System by AMF

A functional mycorrhizal association requires a high degree of coordination between both partners. The fungus has to deal with the plant's immune system, contend with the defense mechanisms and overcome them for successful colonization of the host (Kloppholz et al., 2011; Zamioudis and Pieterse, 2012). Once established, the plant has to regulate the level of fungal proliferation within the roots to prevent excessive colonization and carbon drainage, thus maintaining the interaction at mutualistic levels. For example, under conditions of high exogenous phosphate supply, the plant actively inhibits proliferation of the fungus within the roots (Breuillin et al., 2010). Similarly, plants possess a feedback system that prevents excessive colonization over a critical threshold, a phenomenon termed autoregulation of the symbiosis, described initially in the rhizobium-legume symbioses (Vierheilig, 2004; Vierheilig et al., 2008). Mechanistic similarities between the autoregulation of mycorrhization and nodulation and the induction of systemic resistance by beneficial microbes have been pointed out (Vierheilig et al., 2008; Zamioudis and Pieterse, 2012). In summary, from presymbiotic stages and throughout a well-established AM association, plant defense mechanisms are tightly regulated to control the symbiosis. As a side effect, this regulation may directly impact root pathogens.

During the early stages of the interaction, the plant reacts to the presence of AM fungi by activating some defense-related responses that are subsequently suppressed (García-Garrido and Ocampo, 2002; Liu et al., 2003). Before penetration of the roots, the fungus seems to trigger the plant's immune system as a biotrophic pathogen would (Güimil et al., 2005; Paszkowski, 2006). In response to colonization by AMF, a quick but transient increase of endogenous salicylic acid (SA) occurs in the roots (Fig. 2) with a concurrent accumulation of defensive compounds, such as reactive oxygen species, specific isoforms of hydrolytic enzymes, and the activation of the phenylpropanoid pathway (Pozo et al., 1998; Blilou et al., 1999; Dumas-Gaudot 2000; Fester and Hause, 2005; de Román et al., 2011). These reactions are temporally and spatially limited compared to the reaction during plant-pathogen-interactions, suggesting a role in the establishment or control of the symbiosis (Dumas-Gaudot et al., 1996; García-Garrido and Ocampo, 2002). Indeed, SA signaling seems to have a negative effect on AM colonization (de Román et al., 2011; Herrera-Medina et al., 2003), and AM establishment requires inhibition of certain SA-regulated responses (Dumas-Gaudot 2000) as described for other mutualistic symbiosis (Soto et al., 2009). Despite our lack of knowledge on how the AM fungi evade and manipulate the host´s innate immune system, recent studies support that AM fungi can actively suppress SA-dependent defense reactions by secreting effector proteins that interfere with the host's immune system (Campos-Soriano et al., 2010; Kloppholz et al., 2011). Not only SA, but also the level of other phytohormones related to defense, such as JA, ABA, and ET, is altered during the plant interaction with the AM fungus (Hause et al., 2007; López-Ráez et al., 2010b; Ludwig-Müller, 2010). Indeed, as the colonization progresses, the regulation of JA levels gains a central role in the correct functioning of the AM symbiosis (Hause et al., 2002, 2007; Hause and Schaarschmidt, 2009). Since induced resistance is generally manifested only when the AM symbiosis is well established, the changes in signaling associated with a well-established mycorrhiza are likely mediating MIR (Cordier et al., 1998; Slezack et al., 2000; Pozo et al., 2002, Jung et al., unpublished). It should be noted here that the extent of the hormonal changes associated with the symbiosis depends on the AM fungus involved (López-Ráez et al. 2010b; Fernandez and Pozo, unpublished). The differences may correlate with the differential ability of various AMF to induce resistance. The level of protection conferred by the mycorrhization is highly dependent on the fungus engaged in the symbiosis (Pozo et al., 2002; Garmendia et al., 2004; Kobra et al., 2009; Sikes et al., 2009).

The dependence of successful mycorrhization on the control of JA and SA signaling would explain the range of protection conferred by this symbiosis (Pozo and Azcón-Aguilar, 2007). As summarized, AMF plants are more resistant to necrotrophs and chewing insects, aggressors targeted by JA-dependent defense responses, and they are more susceptible to biotrophs, targeted by SA-regulated defenses. This pattern correlates with an activation of JA-dependent defenses and repression of SA-dependent ones in a well-established mycorrhiza. JA signaling leads to the synthesis of toxins and defensive proteins that target physiological processes in the insect, thus reducing insect growth and survival (Howe and Jander 2008). Similarly, JA regulates defense response genes that are effective against necrotrophic microbial pathogens (Pozo et al., 2009). Moreover, JA triggers indirect plant defenses upon herbivory, regulating the emission of volatile blends that attract predators or parasitoids, which then prey on the herbivores (Dicke et al., 2009; Snoeren et al., 2009). Together with altered JA responses, the volatile profile changes in mycorrhizal plants under attack, and they become more attractive to natural enemies like parasitoids

(Guerrieri et al., 2004; Rapparini et al., 2008; Fontana et al., 2009; Leitner et al., 2010; Schausberger et al., 2011).

Priming of JA-dependent Defenses in Mycorrhiza-Induced Resistance

The induction of resistance (IR), does not necessarily require direct activation of defense mechanisms, but can result from a sensitization of the tissue upon appropriate stimulation to express basal defense mechanisms more efficiently after subsequent pathogen attack. This priming of the plant's innate immune system is common upon interaction with beneficial microorganisms, and has important fitness benefits compared to direct activation of defenses (Conrath et al., 2006; Van Hulten et al., 2006; Van Wees et al., 2008). Induction of the primed state usually is associated with a moderate accumulation of defense-related regulatory molecules, such as transcription factors or MAP kinases (Pozo et al., 2008; Beckers et al., 2009; Van Der Ent et al., 2009b). For example, rhizobacteria-induced systemic resistance in *Arabidopsis* is related to priming of JA-dependent responses through the accumulation of MYC2, a transcription factor with a key role in the regulation of JA responses (Pozo et al., 2008).

Examples of primed defense responses in mycorrhizal plants were first observed in root tissues. Mycorrhizal transformed carrot roots displayed stronger defense reactions at sites challenged by *Fusarium* (Benhamou et al., 1994). In tomato, AMF colonization systemically protected roots against *Phytophthora parasitica* infection. Only mycorrhizal plants formed papilla-like structures around the sites of pathogen infection through deposition of non-sterified pectins and callose, preventing the pathogen from spreading further, and they accumulated significantly more PR-1a and basic β-1,3 glucanases than non-mycorrhizal plants upon *Phytophthora* attack (Cordier et al., 1998; Pozo et al., 1999, 2002). Priming for callose deposition also was reported to underlie protection against *Colletotrichum* in cucumber (Lee et al., 2005). Similarly, mycorrhizal potatoes showed amplified accumulation of the phytoalexins rishitin and solavetivone upon *Rhizoctonia* infection, whereas AMF alone did not affect the levels of these compounds (Yao et al., 2003). Recently, primed accumulation of phenolic compounds in AM date palm trees also has been related to protection against *F. oxysporum* (Jaiti et al., 2007), and priming has been involved in mycorrhizal induction of resistance against nematodes (Li et al., 2006; Hao et al., 2012).

However, the primed response is not restricted to the root system. Recently, we have shown priming of defenses also in shoots of mycorrhizal plants (Pozo et al., 2010). AM symbiosis induced systemic resistance in tomato plants against the necrotrophic foliar pathogen *Botrytis cinerea*. While the amount of pathogen in leaves of mycorrhizal plants was significantly lower, the expression of some jasmonate-regulated, defense-related genes was higher in those plants (Pozo et al., 2010, Jung et al., unpublished). A primed response of JA-dependent defenses was confirmed by transcript profiling of leaves after exogenous application of JA, since JA-responsive genes were induced earlier and to a higher extent in mycorrhizal plants, particularly in those colonized by *G. mosseae* (Pozo et al., 2009). A similar primed response was observed in mycorrhizal tomato leaves upon mechanical wounding or caterpillar feeding, both responses known to be regulated by JA, thus underscoring the importance of the JA signaling pathway in MIR (Fig. 3). The use of tomato mutants impaired in JA signaling has confirmed that JA is required for mycorrhiza induced resistance against *Botrytis* (Martinez-Medina et al., unpublished), confirming that MIR is similar to the well-studied rhizobacteria-induced systemic resistance (ISR) in *Arabidopsis* and requires a functional JA signaling pathway for the efficient induction of resistance (Pieterse et al., 1998). The JA signaling pathway also is required for rhizobacteria-mediated ISR in tomato (Yan et al., 2002), and for the induction of resistance by the beneficial fungi *Trichoderma* and *Piriformospora* (Shoresh et al., 2005; Stein et al., 2008). Recently, MIR against the rice blast fungus *Magnaporthe grisea* has been demonstrated, and the resistance seems to rely on both the systemic activation of genes with a regulatory role in host immunity, and the priming for stronger expression of defense effector genes during pathogen infection (Campos-Soriano et al., 2012).

In addition to this priming effect on above-ground tissues, the AMF hyphal network may even extend the induction of resistance to neighboring plants, acting as a plant-plant underground communication system (Song et al., 2010). Song and co-authors showed that healthy "receiver" plants

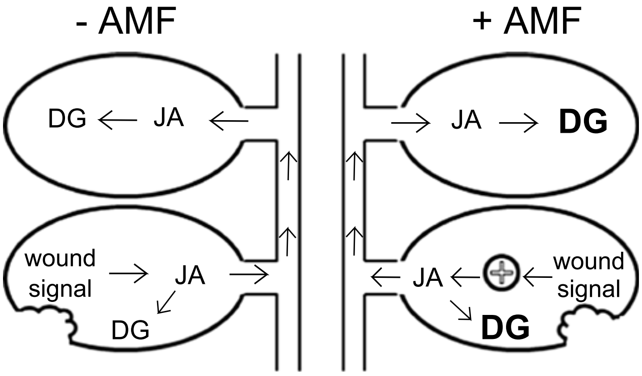

Fig. 3 Priming of jasmonate-dependent wound signaling in the shoots. In non-mycorrhizal plants (− AMF) the initial wound signal activates the JA-dependent pathway that leads to the activation of defense genes (DG) in local and systemic tissue. In mycorrhizal plants (+ AMF) the response to the wound signal is amplified leading to a primed defense response

activate JA-regulated, defense-related genes when neighboring "donor" plants, connected via a common mycorrhizal network in the soil, were infected by the foliar pathogen *Alternaria solani*. According to this finding, plants can 'eavesdrop' on signals from the pathogen-challenged neighbors to activate defenses before being attacked themselves (Song et al*.,* 2010). With regard to this, since mycorrhizal establishment alters the volatile emission, and volatiles have been shown to prime distal plant parts or even neighboring plants for a faster induction of defense responses (Heil and Ton, 2008), it remains to be determined if changes in volatiles in mycorrhizal plants also prime neighboring plants for efficient activation of defense against attackers.

Summary and Outlook

Arbuscular mycorrhizas significantly impact the host plant interaction with multiple organisms. Even though the individual outcome always depends on the AMF-plant-attacker combination, protective effects against deleterious organisms have been described for many interactions (Fig. 1). Experimental evidence confirms that this protection is based not only on improved nutrition or local changes within the roots and rhizosphere, but that priming of plant immunity plays a major role in Mycorrhiza-Induced Resistance. Although the molecular basis for the regulation of plant defenses and the priming of the plant immune system during mycorrhization remains mostly unknown, a prominent role of jasmonate signaling has been confirmed. The great majority of land plants form arbuscular mycorrhizas, thus, unveiling the principles behind a successful symbiosis and the functional interplay between plant and fungus is of major interest. The identification of defense regulatory elements that may operate in priming of plant defenses in mycorrhizal plants may have important practical implications regarding the effectiveness of AMF in the biological control and integrated management of pests and diseases.

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REVIEW ARTICLE

Volatile Mediated Interactions Between Bacteria and Fungi in the Soil

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Abstract Soil is one of the major habitats of bacteria and fungi. In this arena their interactions are part of a communication network that keeps microhabitats in balance. Prominent mediator molecules of these inter- and intraorganismic relationships are inorganic and organic microbial volatile compounds (mVOCs). In this review the state of the art regarding the wealth of mVOC emission is presented. To date, ca. 300 bacteria and fungi were described as VOC producers and approximately 800 mVOCs were compiled in DOVE-MO (database of volatiles emitted by microorganisms). Furthermore, this paper summarizes morphological and phenotypical alterations and reactions that occur in the organisms due to the presence of mVOCs. These effects might provide clues for elucidating the biological and ecological significance of mVOC emissions and will help to unravel the entirety of belowground, volatile-wired' interactions.

Keywords Bacteria · Fungi · Soil · Volatiles · Volatile mediated interactions

Introduction

Inter- and intra-organismal communication strategies are symbolized by the three monkeys: the deaf, the mute, and the blind. Interestingly, one major communication path was not featured: the sense of smell. This is surprising since the sense of smell is well-established in many animals and plants. Vertebrates and invertebrates are able to detect minute amounts of volatiles even over very long distances; plants use volatiles to communicate with their pollinators as well as with plants of the same species or other plants (Baldwin et al., 2006; Dobson, 2006; Heil and Walters, 2009) (Fig. 1). The infochemicals used for these inter- and intra-organismal interactions are low molecular mass compounds with high vapor pressures, low boiling points, and a lipophilic character. All of these features facilitate evaporation. Consequently, these compounds disperse easily in the atmosphere and thus play essential biological/ecological roles in aboveground habitats. It was only recently recognized that belowground organisms are also opulent volatile producers and emitters. Therefore, a new research area focuses on volatile-based interactions in the soil. Here, we first describe the habitat soil with its characteristic structural prerequisites in relation to volatile-based communications. Then, we present a summary of volatile emissions of microbes (bacteria and fungi). In the final section, we discuss volatile-based bacterial and fungal interactions.

The Habitat Soil

The tremendous diversity of the bacterial and fungal kingdoms is paralleled by the heterogeneity of habitats these organisms are able to occupy. They appear ubiquitously around the world, successfully colonizing ecological niches and microhabitats (Dighton, 2003; Hawksworth and Mueller, 2005; Gasch, 2007). One of the major habitats for fungi and bacteria is soil, where they occur as free living organisms on the soil surface, in the soil core, or in association with belowground parts of living plants or organic material derived from dead plants and animals (Forster, 1988). Soil itself is a complex blend of weathered minerals and organic material mixed with biota. Fungi and bacteria hereby play a substantial role in the

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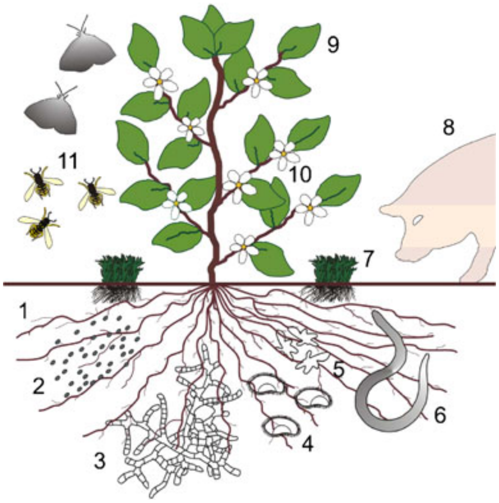

Fig. 1 Schematic presentation of organisms involved in volatile interactions above- and belowground (drawn by Marco Kai). 1 plant root, 2 bacteria, 3 fungi, 4 ciliates, 5 amoeba, 6 nematodes, 7 moos, 8 wild boar, 9 plant leaves, 10 plant flowers, 11 insects

decomposition and breakdown of organic as well as inorganic materials, respectively (Dighton, 2003). These biomineralization processes contribute substantially to soil production, generating a continuous flow of nutrients for plant primary production. Therefore, functional soils should be regarded as a balanced complement of abiotic (mineral and organic) and biotic components (Nakas and Klein, 1980; Dighton, 2003). As part of the microbiotic soil community, fungi and bacteria form dynamic and enduring communities that are integrated into even more complex microecosystems, or they arise as transient communities to secondarily colonize substrates as long as degradable nutrients are available.

Soil Properties Influence Microenvironments Belowground

Microbial colonization of soil is determined mainly by its physicochemical properties (Dequiedt et al., 2011). These properties are influenced by texture, carbon content, and microstructure, which in turn affect the formation of macroaggregates and subsequently soil parameters such as porosity or air and water content. Soil texture is determined by its inorganic components and describes the proportional distribution of mineral particle sizes: sand (0.05–2 mm), silt (2–50 µm), and clay (<2 µm) (Cehnu and Stotzky, 2002; Brown, 2003; Conklin, 2005; Schafer, 2006). Texture, mineral composition, and particle shape give rise to certain particle arrangements (microaggregates) that determine soil microstructures (Cehnu and Stotzky, 2002; Alekseeva, 2007). These microstructures and the presence of organic matter contribute to the assembly and stabilization of macroaggregates >0.25 mm in size (Forster, 1988; Ranjard and Richaume, 2001). As a result, a complex network of void spaces is formed in soil, i.e., soil pores that can account for up to 50 % of the total soil volume (Ranjard and Richaume, 2001; Conklin, 2005; Standing and Killham, 2007). Their ability to retain water varies with their size and shape, so they are filled with different amounts of water and air. Depending on the air and water content, the chemical composition of aggregates, and the circulation within the pore network, numerous heterogenic microenvironments for microbial life are created. These vary in nutrient supply, aeration, availability of water, ionic composition, minerals, pH, redox potential, and surface composition (Forster, 1988; Ranjard and Richaume, 2001; Nannipieri et al., 2003).

Microhabitats Belowground

Microorganisms congregate in soil pores that provide a suitable microenvironment. Bacteria rely on the presence of organic and inorganic solutes in the aqueous phase of pores and on particle surfaces. The heterogeneity of these various microhabitats is probably the reason for the huge bacterial diversity in soil. Although the number of bacterial cells per gram of soil can easily exceed 10^{10} and estimates of the numbers of different species range from 10^3 to 10^5, only a rather small proportion of soils is actually colonized by bacteria (Gans et al. 2005; Roesch et al. 2007; citations in Heuer and Smalla 2012). Bacteria may occur as free living organisms, but are usually attached to solid surfaces as scattered individual cells, microcolonies, or biofilms. Fungi inhabit the same locations but other pore sizes. Water saturated micropores (Ø<10 µm) are reserved for bacterial communities, where they escape predation and the effects of fungal antibiotics. Because of their size, fungi settle in macropores (Ø>10 µm) found between and within macroaggregates. In addition, fungal hyphae can extend through aerated water-unsaturated pores to reach new pores and exploit new nutrient resources (Forster, 1988; Cehnu and Stotzky, 2002). The latter is especially important since soil in its entirety represents a nutrient-depleted habitat for microorganisms. Consequently, microorganisms aggregate near any suitable nutrient source, which creates colonization hotspots. Therefore, bacteria and fungi have to compete for the same resources and undergo interspecies interactions. On the macroscale, plant litter like dead leaves, stems, roots, wood, and bark as well as animal remains and fecal material are important sources of biodegradable organic material, while on the microscale cell-wall remains, lipids, polysaccharides, proteins, DNA and RNA, and metabolites contribute to temporary microhabitats (Forster, 1988; Nannipieri et al., 2003). The most lively and enduring microhabitat is the living plant root, which releases a wide variety of soluble, insoluble, or volatile metabolites that attract an exceptionally dense and diverse population of microbiota, including bacteria and fungi (Koske and Gemma, 1992; Chen et al., 2004; Gregory, 2006; Brimecombe et al., 2007; Nannipieri et al., 2007; Hussain and Hasnain, 2011). Bacteria adhere to the root surface itself (rhizoplane) and colonize a narrow soil zone around the plant root (rhizosphere) (Lenc et al., 2011). They benefit from a constant flow of organic substrates, but in return promote plant growth by providing soluble inorganic nutrients and producing growth-promoting factors (Brimecombe et al., 2007; Nannipieri et al., 2007; Compant et al., 2010). A special role is attributed to antagonistic bacteria, which are able to suppress the growth of various plant pathogenic fungi (Bhattacharyya and Jha, 2011). Mycorrhizal fungi (see Jung et al., 2012, this issue) also benefit from nutrients supplied by the plant root. More than 95 % of short roots of most terrestrial plants are colonized by symbiotic fungi, and these mycorrhizal fungi are surrounded by complex microbial communities. So called mycorrhiza helper bacteria (MHB) support mycorrhiza formation (Frey-Klett et al., 2007; Bonfante and Anca, 2009; Rigamonte et al., 2010). In addition, plant roots not only host beneficial but also attract detrimental organisms such as phytopathogens, which may harm plants and microbiota as well. Therefore, mycorrhizal fungi, their associated bacteria as well as rhizobacteria have to deal with a very complex and competitive rhizomicrobial milieu (Anderson, 1992; Bianciotto et al., 1996; Miransari, 2011). Bacteria and fungi closely intermingle in the mycorrhizosphere and mutually influence survival and colonization success as well as pathogenesis and virulence (Wargo and Hogan, 2006; Minerdi et al., 2008).

Volatiles as Medium for Interactions Belowground

Factors that regulate the dynamics and balance of symbiosis, cooperation, competition, and also coexistence in microbial communities have been investigated intensively. Phenomena like quorum-sensing and quorum-quenching (see Hartmann and Schikora, 2012, this issue), the impact of rhizobacterial and fungal antibiotics, effector molecules, and excreted enzymes have been recognized as effective regulatory principles (Walker et al., 2003, 2004; Chernin et al., 2011). The possible role of volatiles in bacterial-fungal interactions has been neglected for many years despite earlier reports on effective microbial volatiles (Stotzky and Schenk, 1976; Koske and Gemma, 1992). Prerequisite for volatile effectiveness is their release, emanation and distribution, and their perception by a target organism. This is ensured by the physicochemical properties of volatiles (low molecular weight, high vapor pressure, low boiling point), which facilitate distribution even over long distances (Farmer, 2001; Baldwin et al., 2006; Heil and Ton, 2008). However, does this also occur in soils? Yes, it does. Volatile distribution belowground takes place by diffusion and advection (Minnich and Schumacher, 1993). Volatiles can move through the network of soil pores since they are active in both gas and liquid phases and capable of revolatization after passing through water-saturated pores (Koske and Gemma, 1992; Aochi and Farmer, 2005; Asensio et al., 2008). However, due to their high vapor pressure, volatiles move primarily by vapor diffusion (Minnich and Schumacher, 1993). These processes are all influenced by inherent chemical properties of the volatile itself and physicochemical properties of the surrounding soil, which affect adsorption, desorption, and degradation. Adsorption/desorption depends on the polarity of the compound, the soil texture and spatial architecture, and the presence of water. On the microscale, increasing humidity reduces the adsorption of nonpolar volatiles to mineral surfaces; on the macroscale, nonpolar volatiles are increasingly sorbed by organic matter in moist or wet soils (Minnich and Schumacher, 1993; Ruiz et al., 1998; Aochi and Farmer, 2005; Insam and Seewald, 2010). Volatile compounds also are amenable to biodegradation. Owen et al. (2007) found rapid degradation of geraniol in the rhizosphere of *Populus tremula*, an observation they attributed to the activity of soil microorganisms. However, compared to compounds solely soluble in water, volatiles are less likely to be quickly biodegraded (Koske and Gemma, 1992). Mineral surfaces may serve as catalysts for chemical reactions that contribute to abiotic degradation. Highly specific clay surfaces react with volatiles that carry polar functional groups. Furthermore, volatiles also may be exposed to free-radical oxidation (Minnich and Schumacher, 1993; Insam and Seewald, 2010). Measurements of volatile exchange rates have revealed low volatile emission from soil, supporting the assumption that soil acts as a volatile sink (Stotzky and Schenck, 1976; Asensio et al., 2007).

Microbial Volatile Emission

A large number of bacterial species presently are known, and it is estimated that this number could reach a million (10^6). While many microorganisms have been isolated from aboveground habitats (i.e., plants, human skin and intestines, animals, and refuse, sewage, and aquatic habitats), a rich source of bacteria is the terrestrial and belowground biotope. Metagenomic approaches have demonstrated that the microbial diversity is larger in soils than in marine sediments or aquatic habitats (Will et al., 2010; Daniel, 2011). The capacity of bacteria and fungi to decompose, mineralize, and accumulate organic matter is extraordinary and has a significant impact on the carbon, nitrogen, phosphate and sulfur biogeochemical cycles (Naeem, 1997). Some of the metabolized compounds are emitted as volatile products that are readily utilized by other organisms of the microbial food chain or released into the underground

Table 1 Producers and users of microbial volatiles

Producer/emitters →	compound	→ user/receiver	function
Inorganic			
No microorganism known	CO	Carboxydotrophic bacteria (*Hydrogenomonas carboxydovorans*, *Selberia carboxyhydrogena Oligotropha carboxidovorans Carboxydothermus hydrogenoformans*)	Electron donor, carbon source
Heterotrophic microbes	CO_2	Chemolitho (hydrogen-, sulfur-, ammonia-, nitrite-. Fe^{2+}-oxidizing)- and oxygenic (cyanobacteria) and anoxygenic (Rhodospirillaceae, Chromatiaceae, Chlorobiaceae, Chloroflexaceae) photoautotrophic bacteria	Carbon source
		Methanogenic archaea (*Methanobacterium ruminatium, M. thermoautrophicum*) and homoacetogenic bacteria (*Clostridium aceticum, C. ljungdahlii*)	Electron acceptor (methane or acetate production)
Facultative and obligate anaerobic bacteria (clostridia, enterobacteria)	H_2	Chemolitho (hydrogen-oxidizing)- and anoxygenic (Rhodospirillaceae, Chromatiaceae, Chlorobiaceae, Chloroflexaceae) photoautotrophic bacteria	Electron donor
		Methanogenic archaea (*Methanobacterium ruminatium, M. thermoautrophicum*) and homoacetogenic bacteria (*Clostridium aceticum, C. ljungdahlii*)	
Cyanobacteria (*Synechococcus, Synechcystis*)	O_2	Aerobic microorganisms	Electron acceptor
Proteolytic clostridia and some aerobic chemoorganotrophic proteobacteria (*Serratia odorifera* 4Rx13, *Serratia plymuthica* HRO C48, *Pseudomonas fluorescens* L13-6-12, *Pseudomonas* trivialis 3Re2-7, *Stenotrophomonas rhizophila* P69, *Xanthomonas campestris* pv. *vesicatoria* 85-10)	NH_3 (NH_4^+)	Many microorganisms Ammonia-oxidizing bacteria (Nitrosomonas, Nitrosospira, Nitrosococcus)	Nitrogen source, olfaction, antibiotic resistance, toxic compound, electron donor
Ammonia-oxidizing bacteria (*Nitrosomonas, Nitrosospira, Nitrosococcus*)	NO_2^-	Nitrite-oxidizing bacteria (Nitrobacter, Nitrospina, Nitrococcus)	Electron donor, nitrogen source
Denitrifying bacteria (*Alcaligenes faecalis, Bacillus licheniformis, Paracoccus denitrificans, Pseudomonas stutzeri, Thiobacillus denitrificans*)	N_2	Nitrogen-fixing bacteria and archaea (*Rhizobium, Bradyrhizobium, Azorhizobium, Frankia, Klebsiella, Clostridium, Methanosarcina, Methanospirillum*)	Nitrogen source
Some proteobacteria (*Pseudomonas fluorescens, P. trivialis, Chromobacterium, Rhizobium*)	HCN		Defense compound, quorum sensing
Sulfate-reducing bacteria (*Desulfovibrio, Desulfomonas, Desulfotomaculum*)	$H_2S/S/SO_3^{2-}$	Chemolitho (sulfur-oxidizing)- and anoxygenic (Chromatiaceae, Chlorobiaceae) photoautotrophic bacteria	Electron donor, amino acid biosynthesis, defense compound
Organic			
Methanogenic archaea	CH_4	Obligate methylotrophic bacteria	Electron donor, carbon source
Many bacteria, see Table 2	Alkanes, alkenes	Aerobic microorganisms	Carbon source
Clostridium spp., *Pseudomonas* spp., *Streptomyces* spp.	ethylene		
Yeast; several bacteria, see Table 2	CH_3OH		

Table 1 (continued)

Producer/emitters →	compound	→ user/receiver	function
		Obligate and faculative methylotrophic bacteria methanogenic archaea	Electron donor, carbon source, electron acceptor
Yeast, facultative and obligate anaerobic bacteria (e.g. clostridia, enterobacteria, lactic acid bacteria); several bacteria and fungi, see Tables 2 and 3	C_2H_5OH	Heterotrophic bacteria (acetic acid bacteria, *Clostridium kluyveri*)	Electron donor, carbon source
Clostridium spp., *Bacillus* spp., *Lactobacillus* spp., *Salmonella* spp., *Shigella* spp., *Streptomyces* spp.; many other bacteria and fungi, see Tables 2 and 3	Butanol	Heterotrophic bacteria	Electron donor, carbon source
Many bacteria and fungi, see Tables 2 and 3	Low molecular mass alcohols	Heterotrophic bacteria	Carbon source
Enterobacteria, bacilli, clostridia	Acetoin, 2,3-butanediol	Heterotrophic bacteria	Electron donor, carbon source
	CH_2O	obligate and faculative methylotrophic bacteria	electron donor, carbon source
Many bacteria and fungi, see Tables 2 and 3	Low molecular mass aldehydes and ketones	Heterotrophic bacteria	Carbon source
Clostridia and other bacteria, see Table 2; fungi see Table 2	Acetone	Heterotrophic bacteria	Electron donor, carbon source
Facultative and obligate anaerobic bacteria (clostridia, enterobacteria, lactic acid bacteria), many bacteria and fungi, see Tables 2 and 3	$CHOOH$, CH_3COOH, CH_3CH_2COOH, $CH_3CH_2CH_2COOH$, low molecular mass acids	Heterotrophic bacteria and fungi	Electron donor, carbon source
Alcaligenes spp., *Bacillus* spp., *Pseudomonas* spp., *Stenotrophomonas* spp.; many other bacteria see Table 2, few fungi see Table 3	Methylamine and other amines	Obligate and faculative methylotrophic bacteria methanogenic archaea	Electron donor, carbon source
Streptomyces spp.; see Table 2	Geosmin		
Alcaligenes spp., Chondromyces spp., Paenibacillus spp., Serratia spp.; many other bacteria see Table 2	Pyrazines		
Enterobacteria, *Pseudomonas* spp.	Indole		Signaling (indirect?)
Streptomyces spp., *Bacillus* spp., *Pseudomonas* spp., *Tuber* spp.; many other bacteria and fungi, see Tables 2 and 3	Dimethyldisulfide, trimethyldisulfide	Marine sulfur-oxidizing bacteria (*Thiobacillus thioparus*) obligate and faculative methylotrophic bacteria *(Hyphomicrobium)*	Carbon and energy source
Gram negative bacteria (*Pseudomonas fluorescens, Serratia liquefaciens*)	N-acyl-l-homoserine lactones (AHL) (e.g. C4-HSL,C6-HSL, C10HSL)	Gram negative bacteria (*Pseudomonas aeruginosa*, *Agrobacterium tumefaciens*	Quorum sensing signal > biofilm formation
Gram positive bacteria *Streptomyces*	Gamma-butyrolactones	Gram positive bacteria	Quorum sensing signal
Condromyces spp., *Leuconostoc* spp., *Roseobacter* spp.,*Lactobacillus* spp., *Serratia* spp.; many other bacteria, see Table 2	ß-phenylethanol		
	Aromatic compounds	Aerobic and anaerobic bacteria	Carbon source
Serratia odorifera 4Rx13, *S. plymuthica* HRO-C48	Sodorifen		

The table presents examples of microorganisms that synthesize or use inorganic or organic volatile compounds. Summarized from G. Gottschalk, 1986; Fuchs. 2007; and DOVE-MO (Kalderas, 2011) habitat (Table 1). Soil microorganisms produce large quantities of highly diverse volatiles (Stotzky and Schenck, 1976; Linton and Wright, 1993; Leff and Fierer, 2008; Insam and Seewald, 2010 and citations therein). Volatile metabolites also are produced by the root system of plants, but in this review these sources will not be considered. Instead, the focus lies on bacterial and fungal volatile emissions and uptakes (Kesselmeier and Staudt, 1999; Wenke et al., 2009). The volatile compounds can be of organic (volatile organic compounds, VOCs) or inorganic nature, both presumably important for this habitat and capable of influencing organismic communities (McNeal and Herbert, 2009). The functions of the volatiles are diverse, e.g., i) they play a role in the food chain of the microbial loop because they are assimilated and incorporated into organic matter (bioconversion), ii) they influence physiological processes (e.g., laccase activity, nitrification, nitrogen mineralization), iii) they function as electron acceptors or donors to support metabolic reactions, iv) they play a role in quorum sensing/quenching, v) they act as defense compounds, vi) they are used as communication signals, or vii) their functions remain so far elusive (Table 1).

Volatiles Emitted from Bacteria

Inorganic Volatiles

Some producers and users of inorganic volatiles are summarized in Table 1, which is a brief extract from Gottschalk (1986) and Fuchs (2007). **Carbon dioxide** is a major inorganic volatile produced by all heterotrophic living organisms, and indeed much of the CO_2 in the atmosphere originates from the huge microbial populations on earth, in both soil and aquatic habitats. Atmospheric CO_2 is assimilated primarily by plants and oxygenic and anoxygenic phototrophic bacteria (cyanobacteria, *Rhodospirillaceae* [purple nonsulfur bacteria], *Chromatiaceae* [purple sulfur bacteria], *Chlorobiaceae* [green sulfur bacteria], and *Chloroflexaceae* [green nonsulfur bacteria]). The characteristic Calvin reactions and enzymes also are present in soil bacteria, such as *Rhodospirillum rubrum, Thiobacillus intermedius, Ralstonia eutrophus*, *Pseudomonas facilis*, to name a few. Chemolithotrophic microorganisms use ATP and the reducing power of inorganic substrates for the reduction of CO_2. CO_2 also is used by methanogenic bacteria such as *Methanobacterium ruminatium* and *Methanobacterium thermoautrophicum* for CH_4 production (Gottschalk, 1986).

Anthropogenically released **carbon monoxide** results from incomplete reduction of wood and polymers of dead organic material, while microbial CO production is unknown. Aerobically grown *Hydrogenomonas carboxydovorans* and *Selberia carboxyhydrogena* can live on CO by oxidizing it to CO_2. Some bacteria (e.g., *Rhodospeudomonas sphaeroides, Methylosinus, Methylocystis*) use the serine-isocitrate lyase pathway to form oxaloacetate from phosphoenol pyruvate (PEP) and CO_2 (PEP carboxylase). As a result of this pathway, acetyl-CoA and finally succinate are formed from CH_2O and CO_2. Chemolithotrophic and phototrophic bacteria have in common the formation of cell material via CO_2 reduction by using the reducing power from inorganic compounds. Energy sources can be H_2, sulfide, ammonia, or nitrite.

Hydrogen is formed under anaerobic conditions during the fermentation of carbohydrates to short-chain fatty acids by *Clostridium* spp., *Enterobacteriaceae* (e.g., *Escherichia, Salmonella, Shigella*) and others. A group of chemolithotrophic bacteria (hydrogen-oxidizing bacteria), anoxygenic phototrophic bacteria, as well as methanogenic archaea utilize H_2 as an electron donor.

Well-known volatile-dependent soil bacteria are the free-living and symbiotic **nitrogen**-fixing organisms. The latter are, for example, *Rhizobium* spp. and *Frankia* spp., and exist in partnerships with plants. These bacteria form bacteroids, and consequently, root nodules develop. The product of the nitrogenase is **ammonia**, which is usually not released but is efficiently incorporated into organic compounds by glutamate dehydrogenase, glutamine synthetase, and glutamate synthase. Soil-living clostridia (*Clostridium* spp.) and other bacteria (e.g., *Peptococcus anaerobicus*) ferment amino acids and nucleotides and live from these recycled carbon skeletons as well as ammonia. Recently, it was shown that *Serratia, Pseudomonas, Stenotrophomonas*, and *Xanthomonas*, when grown on complex media (NB or LB), emitted gaseous ammonia (or amines), which was detected in the headspace with Nessler's reagent (Kai et al., 2010; Weise et al., 2012, Weise and Piechulla unpublished). Gaseous ammonia released from bacteria can modify, e.g., the antibiotic resistance of *E. coli* to tetracycline (Bernier et al., 2011). Apparently, increased intracellular polyamine levels alter the membrane permeability to antibiotics as well as resistance to oxidative stress. Another recent publication showed that ammonia could be sensed by *Bacillus licheniformis*, which was considered to be a first indication of bacterial olfaction (Nijland and Burgess, 2010). Although the nitrogen supply is usually a limiting factor in soil, it cannot be excluded that NH_3 emission may occur in nature under confined protein-rich growth conditions (e.g., decomposition of carcasses, lysis of large microbial populations or plant materials, or land spreading of whey in agriculture). The amounts as well as the ecological consequences have not been investigated.

Denitrifying bacteria release nitrogen during respiration and reduction of nitrate (in some cases N_2O instead of N_2 is released). The group of nitrogen-evolving bacteria is quite diverse, including *Alcaligenes faecalis, Bacillus licheniformis, Paracoccus denitrificans*, and *Pseudomonas stutzeri*.

Most soil microorganisms use sulfate as their principal sulfur source, and the intrinsic enzyme system reduces sulfate to sulfide (sulfate assimilation). However, in anaerobic regions in the soil, sulfate is used by *Desulfovibrio, Desulfomonas, Desulfuromonas*, and *Desulfotomaculum* as a terminal electron acceptor, and consequently **hydrogen sulfide** is formed and released (dissimilatory sulfate reduction). The toxic end product H_2S is used by chemolithotrophic bacteria as electron acceptor, e.g., *Thiobacilli*, and H_2S can also be incorporated into O-acetylserine, an intermediate of amino acid biosynthesis. Furthermore, it also has been shown that H_2S production in soil is due to the presence of cysteine (Morra and Dick, 1991). Only recently it was demonstrated that H_2S production acts as a defense mechanism that protects bacteria from antibiotics (Shatalin et al., 2011).

The release of **HCN** from bacteria varies in different species (Stotzky and Schenck, 1976). *Pseudomonas* spp. (e.g., CHA0), *Chromobacterium* and *Rhizobium* typically emit this toxic inorganic volatile, while defective mutants (e.g., CHA207) do not (Blumer and Haas, 2000; Pessi and Haas, 2000; Kai et al., 2010; Blom et al., 2011b). Hydrogen cyanide inhibits several metal-containing enzymes, most significantly the cytochrome c oxidase of the respiratory chain. Therefore, this volatile can be toxic for most aerobic organisms living in the same habitat as Pseudomonades. It was reported that both the RHI/R- as well as the AHL-based quorum sensing system regulate HCN biosynthesis (Winson et al., 1995; Pessi and Haas, 2000). Consequently, bacterial population densities can be controlled by HCN levels.

The distribution and appearance of inorganic gaseous compounds in the soil determine the localization of other soil organisms, e.g., the oxidizers (nitrification) of ammonium occur in the upper sediment layers, followed by nitrate and sulfide oxidizers. In the deeper anaerobic layers, methanogenic and acetogenic bacteria reside. Many of the gaseous compounds are quickly recycled (e.g., H_2) because producers and utilizers appear in nearby soil zones. Compounds emitted in excess are released into the atmosphere, for example, CO_2, N_2, and in some regions H_2S.

Organic Volatiles (VOCs) (<120 D)

The smallest organic volatile compound is **methane**, the most reduced compound. Its formation is the terminal step in the food chain of methanogenic archaea (Gottschalk, 1986). They utilize CO_2, CH_2O, HCOOH, or CH_3OH and H_2 to synthesize methane. This soil-based methane production is of global importance; for example, tundra and rice fields contribute 40 % of atmospheric methane. In the soil, CH_4 is a good substrate for obligate and facultative methylotrophs, which are often anaerobic organisms that grow in deeper soil layers. Bacterial production of the C_1 volatile **methanol** has been described in *Enterobacteriaceae* such as *Escherichia coli, Shigella flexneri*, and *Salmonella enterica* (Bunge et al., 2008) and in *Xanthomonas campestris* (Weise et al., 2012). Methanol can be metabolized by methylotrophic bacteria including *Hyphomicrobium* species, some *Pseudomonas* species (*P. oxalaticus*), and *Protaminobacter* (Gottschalk, 1986). After an initial conversion into formaldehyde, a condensation with ribulose-5-phosphate forms dihydroxyacetone phosphate in the so–called ribulose-monophosphate cycle in *Methylococcus* and *Methylomonas* species. Yeasts, *Zymomonas mobilis*, lactic acid bacteria, and clostridia form **ethanol** (Gottschalk, 1986). Ethanol together with acetate is a good substrate for *Clostridium kluyveri*. **Butanol** and **acetone** are emitted e.g., by *Clostridium acetobutylicum* when enzymes of this pathway are activated under low pH conditions (Lütke-Eversloh and Bahl, 2011). Butanol also is formed by various microorganisms, and is considered a volatile organic compound (VOC). In the presence of butyrate and e.g., during glucose depletion butanol is a preferred product of butyrate metabolism. Many clostridia reduce acetone to isopropanol. **Acetoin** and **2,3-butanediol** typically are produced during incomplete oxidation by *Bacillus* spp. (Gottschalk, 1986). Formed from pyruvate via α-acetolactate, both compounds are released under glucose abundance and taken up when glucose is depleted. Acetoin and 2,3-butanediol then can serve as a source for ATP production needed during the sporulation process. Butanediol production also is carried out by *Enterobacteriaceae* e.g., *Serratia*, *Enterobacter*, and *Erwinia*. Small molecular weight acids such as formate, acetate, propionate and butyrate are typical mixed acid fermentation products synthesized by *Enterobacteriaceae*, *Clostridia*, *Propionibacteria*, and e.g., *Megasphaera elsdenii* (Gottschalk, 1986). Small organic acids are utilized by many heterotrophic soil microorganisms.

Volatile Organic Compounds (>120 D) Emitted from Bacteria and Fungi

It is well-known that bacteria emit small molecular weight organic volatiles (<120 D, see above), but the frequent release of other compounds (120 to ca. 300 D) by microorganisms has only recently attracted attention. A literature search allowed the compilation of around 800 VOCs emitted by bacteria and fungi. Most compounds are in the range from 130 to 210 D (Fig. 2). In the 'database of volatiles emitted by microorganisms (DOVE-MO),' all VOC emitting microorganisms were compiled, including those in soil (literature search till December 2010, Kalderas, 2011). Since the origin of the microbes often was not well-documented, or it was difficult to assign microorganisms to a single habitat, we compiled all VOC emitting microorganims in DOVE-MO (Database of volatiles emitted by microorganisms) and present them in alphabetical order (bacteria: Table 2, fungi: Table 3). In total, 671 different VOCs are emitted by 212 bacterial species, and

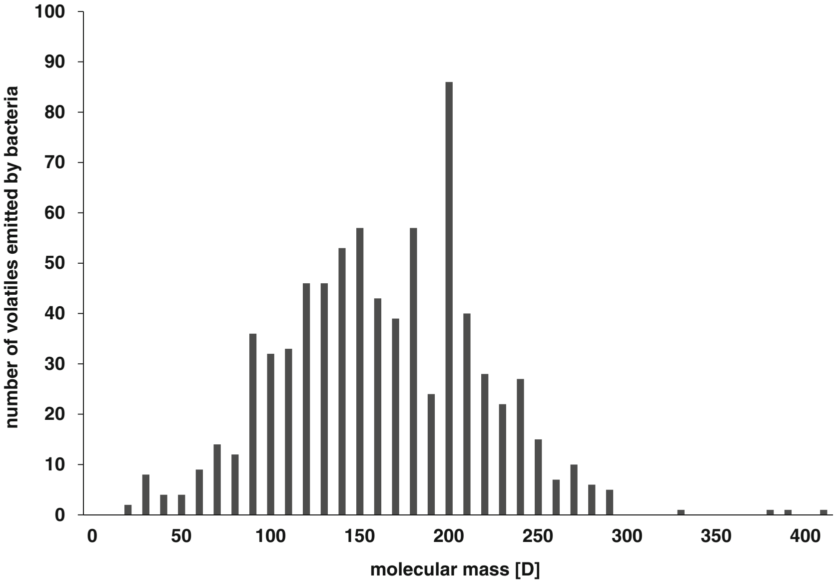

Fig. 2 Distribution of molecular masses of bacterial volatile organic compounds (VOCs)

335 VOCs from 96 fungal species are known. It is expected that future investigations in this new and developing research area will rapidly add organisms and VOCs to this database.

The volatile spectra of the microbes can be simple (<10 VOCs) as well as very complex (>50 VOCs) (e.g., Kai et al., 2007, 2010). Approximately 50 bacterial and ca. 30 fungal species presently are known that emit complex volatile mixtures. The number of detectable volatiles in a species blend increases when various techniques are applied (e.g., dynamic headspace volatile capture in open and closed airflow systems, different trapping materials, solid phase microextraction (SPME), gas chromatography combined with mass spectrometry (GC/MS), proton transfer reaction/mass spectrometry (PTR-MS), selected ion flow tube/mass spectrometry (SIFT-MS), secondary electron spray ionization/mass spectroscopy (SESI-MS), as well as analytical chemistry) (summarized in Wenke et al., 2012). Furthermore, the effects of growth media and conditions on the emission spectra have to be considered (Fiddaman and Rossall, 1994; Kai et al., 2010; Blom et al., 2011a).

The compiled information of volatile-producing microorganisms and their emission profiles was used to search for characteristic VOCs emitted by certain bacterial or fungal genera. The dominant classes of compounds emitted by fungi are alcohols (e.g., isomers of butanol, pentanol, octanol), hydrocarbons, ketones, terpenes, alkanes, and alkenes (Chiron and Michelot, 2005, Table 3). Prominantly emitted VOCs from bacteria are alcohols, alkanes, alkenes, and ketones, followed by esters and pyrazines, lactones, and sulfides (Wenke et al., 2012, Table 2). Some examples are given. *Streptomyces* species are especially rich in sesquiterpenes (Citron et al. 2012) and preferentially emit methylated short-chain alcohols and acids, while *Pseudomonas* species release C9-C16 alkanes/alkenes (Table 2). The product profiles of *Bacteroides* spp. and *Lactobacillus* spp. are rich in various C4 to C16 methylated carboxylic acids, C4 to C14 carboxylic acids, and small methylated alcohols (Table 2). Short-chain and long-chain acids are well-known carbon sources for many microorganisms, but the role of low molecular mass ketones and alcohols in the metabolic food chain is less clear (Table 1). N-acyl-l-homoserine lactones (AHL) are preferentially used as infochemicals (Ryan and Dow, 2008; Dickschat, 2009). Methylamine and other amines serve as good electron donors and carbon sources for many methylotrophic bacteria and methanogenic bacteria. The emission of indole from enterobacteria is well-known, but its ecological relevance is still speculative; an effect in indirect signaling has been indicated (Ryan and Dow, 2008). The sulfur containing compounds dimethyldisulfide (DMDS) and dimethyltrisulfide (DMTS) are often emitted from bacteria and fungi (Tables 2 and 3). While the organic sulfur compounds dimethylsulfide (DMS) and dimethylpropionate (DMSP) play central roles in the global sulfur cycles. This is apparently not the case for DMDS and DMTS (Schäfer et al., 2010). A clear picture on the biological or ecological relevance of the latter compounds is still missing since contrasting results have been obtained. DMDS had inhibitory effects on *Arabidopsis thaliana* in dual culture assays (IC_{50}: 2.5 μmol) (Kai et al., 2010), while in another study it was shown that it could protect plants against fungal pathogens due to the induction of systemic resistance (Huang et al., 2012).

Prominent in bacterial emission profiles are pyrazines and β-phenylethanol. However, their biological functions are presently elusive. Even less understood is the biological and ecological relevance of the emission of extraordinary structures such as the terpene geosmin and sodorifen (Gerber and Lechevalier, 1965; Dickschat et al.,

Table 2 Compilation of VOC producing bacteria

Species	Tax ID (NCBI)	Volatile Synonym	References
Acinetobacter calcoaceticus	471	Sulfoacetaldehyde	Schulz and Dickschat, 2007
Actinobacillus actinomycetemcomitans	714	Acetic acid	Kurita-Ochiai et al., 1995
Aeromonas veronii	654	Dimethylselenide, Dimethyldisulfide, Methaneselenol, Dimethylselenenylsulfide	Schulz and Dickschat, 2007
Alcaligenes faecalis	511	Acetamide, Benzaldehyde, Phenylacetaldehyde, Methanamine, Benzothiazole, Methylpyrazine, 1-Butanamine, Dodecane, Dimethyldisulfide, Nonadecane,1-Decene, 2,5-Dimethylpyrazine	Zou et al., 2007
		Methanethiol, Dimethylsulfide	Schulz and Dickschat, 2007
Alcaligenes spp.	507	3-(Methylsulfanyl)propan-1-ol, Dimethyldisulfide, 2-Methylmercaptoethanol	
Alteromonas spp.	226	Methyliodide	
Anabaena spp.	1163	Geosmin	
Arctic sea ice associated bacterium ARK10141	196850	Tridecan-2-one, Dimethyldisulfide, Dimethyltrisulfide, Trimethylpyrazine, Hexadecan-2-one, 2,5-Dimethylpyrazine, Pentadecan-2-one, Tetradecan-2-one, 13-Methyltetradecan-2-one, 13-Methyltetradecan-3-one	Dickschat et al., 2005c
Arctic sea ice associated bacterium ARK10146	196852	Benzaldehyde, Dimethyldisulfide, Dimethyltrisulfide, Trimethylpyrazine, 2,5-Dimethylpyrazine, Pentadecan-2-one, Tetradecan-2-one, 13-Methyltetradecan-2-one, 13-Methyltetradecan-3-one	
Arctic sea ice bacterium ARK10044	196844	Tridecan-2-one, Hexadecyl acetate, Trimethylpyrazine, Hexadecan-2-one, 2,5-Dimethylpyrazine, Pentadecan-2-one, Tetradecan-2-one, 13-Methyltetradecan-2-one	
Arctic sea ice bacterium ARK10063	196865	Benzaldehyde, Menthol, Camphor, Clovene, para-Menth-1-en-4-ol, alpha-Terpineol, Trimethylpyrazine, Dihydroactinidiolide, 2,5-Dimethylpyrazine, Pentadecan-2-one, Borneol, Isolongifolene, beta-Ionone 5,6-epoxide, beta-Ionone 5,6-epoxide, beta-Caryophyllene, 13-Methyltetradecan-2-one	
Arctic sea ice bacterium ARK10223	196854	Benzaldehyde, Tridecan-2-one, Trimethylpyrazine, 2,5-Dimethylpyrazine, Pentadecan-2-one, Tetradecan-2-one, 13-Methyltetradecan-2-one	
Arctic sea ice bacterium ARK10267	196855	Benzaldehyde, Benzyl alcohol, Phenol, Camphor, 2-Phenylethanol, Furfural, Acetophenone, Methylpyrazine, Clovene, Calamenene, para-Menth-1-en-4-ol Tridecan-2-one, Tetramethylpyrazine, alpha-Terpineol, Dodecan-2-one, 3-Ethyl-2,5-dimethylpyrazine, 2-Ethyl-3,5-dimethylpyrazine, Trimethylpyrazine, Ethyltrimethylpyrazine, Hexadecan-2-one, 2,5-Dimethylpyrazine, Tetradecan-2-one, Isolongifolene, Cadina-1(10),6,8-triene, Geranylacetone, beta-Caryophyllene, 2,5-Diethyl-3,6-dimethylpyrazine, 13-Methyltetradecan-2-one, 11-Methyldodecan-2-one, 2,6-Diethyl-3,5-dimethylpyrazine	
Arthrobacter globiformis	1665	Phenylacetaldehyde, 2-Phenylethylamine	Schulz and Dickschat, 2007
Arthrobacter nitroguajacolicus	211146	Acetamide, Benzaldehyde, Phenylacetaldehyde, Methanamine, Benzothiazole, Methylpyrazine, 1-Butanamine, Dodecane, Dimethyldisulfide Nonadecane, 1-Decene, 2,5-Dimethylpyrazine	Zou et al., 2007
Azoarcus evansii	59406	Phenylacetate	Schulz and Dickschat, 2007
Bacillus amyloliquefaciens	1390	Acetoin, 2,3-Butanediol	Ryu et al., 2003; Farag et al., 2006
		Acetone Benzaldehyde, 1-Butanol, 2,3-Butanedione, Ethanol, Glyoxylic acid, Methanethiol, 1-Pentanol, Acetylene, Isoprene, 2-Methyl -1-propanol, 2-Butanone, Diethylacetic acid, 2-Methylbutanal, Cyclohexane, Dodecane, 2-Methyl-1-butanol, Ethyl acetate, 3-Methylbutanoic acid, 2-Methylfuran, Hexadecane, 3-Methylbutanal, Dimethyldisulfide, 1-Undecene, Tetrahydro-2,5-dimethylfuran, Undecane, 2-Ethylfuran, Dimethyltrisulfide, 2-Pentylfuran, 3-Methyl-1-butanol, Acetic acid butyl ester, Butanol-3-methyl acetate, 1-Methoxy-3-methylbutane, 2-Hydroxy-3-pentanone, 2,4-Hexadienal	Farag et al., 2006
Bacillus popilliae	78057	N-3-Methylbutylidene-3-methylbutylamine, N-Phenylmethylene-2-methylpropylamine, N-Phenylmethylene-3-methylbutylamine	Schulz and Dickschat, 2007
		N-Isopentylideneisopentylamine	Dickschat et al., 2005b
Bacillus pumilus	1408	n-Hexadecanoic acid, Diethylphthalate, 3-Methyl-1-butanol, oleic acid, 8-Methyl-1-decene, 3,4-Dimethyl-5-hexen-3-ol, (*E*)-2-Octenal, 2,4-Decadienal, (*Z*)-2-Heptenal	Wei-wei et al., 2008

Table 2 (continued)

Species	Tax ID (NCBI)	Volatile Synonym	References
Bacillus simplex	1478	Propanone, Benzaldehyde, Phenol, Benzenacetaldehyde, Propionic acid, 1-Hexadecanol, Phenylethanone, Cyclohexene, Benzene ethanol, Nonane, 2-Undecanone, Decanal, Dodecane, Hexadecane, Dimethyldisulfide, Tetradecane, 2-Nonanone, Terpineol, 2-Octanol, Trimethylpyrazine	Gu et al., 2007
Bacillus spp.	1386	Acetic acid, Acetoin, Isoprene, 3-(Methylsulfanyl)propan-1-ol, Dimethyldisulfide, 2,3,5,6-Tetramethylpyrazine, 2-Methylmercaptoethanol, (2R,3R)-Butane-2,3-diol	Schulz and Dickschat, 2007
		Acetamide, Phenylacetaldehyde, Methaneamine, Benzothiazole, Methylpyrazine, 1-Butanamine, Dodecane, Nonadecane, 1-Decene, 2,5-Dimethylpyrazine	Zou et al., 2007
		2,3-Butanediol, Acetoin	Ryu et al., 2003
		Octanal, n-Hexadecanoic acid, Diethylphthalate, Octadecanoic acid, Heptanol, 2-Pentylfuran, Nonanal, 2-Methyl-7-oxabicyclo[2.2.1] heptane, Oleic acid, (*E*)-2-Octenal, (*E*)-2-nonenal, (*E*)-2-decenal, 2,4-Decadienal, 2-Undecenal, (*Z*)-2-Heptenal	Wei-wei et al., 2008
		Acetone, 2,3-Butanedione, Ethanol, Glyoxylic acid, Methanethiol, 1-Pentanol, Acetylene, Isoprene, 2-Methyl-1-propanol, 2-Butanone, Diethylacetic acid, 2-Methylbutanal, Benzaldehyde, 1-Butanol, 2,3-Butanediol, Acetoin, Cyclohexene, Dodecane, 2-Methyl-1-butanol, Ethyl acetate, 3-Methylbutanoic acid, 2-Methylfuran, Hexadecane, 3-Methylbutanal, Dimethyldisulfide, 1-Undecene, Tetrahydro-2,5-dimethylfuran, Dimethyltrisulfide, 2-Pentylfuran, 3-Methyl-1-butanol, Acetic acid butyl ester, 3-Methyl-butanol acetate, 2-Hydroxy-3-pentanone, 2,4-Hexadienal	Farag et al., 2006
		Benzaldehyde, Propanone, Phenol, 1-Hexadecanol, Benzenacetaldehyde, Propionic acid, Benzeneethanol, Phenylethanone, Cyclohexene, Decanal, Dodecane, Hexadecane, Dimethyldisulfide, Tetradecane, Nonane, 2-Nonananone, 2-Undecanone,Terpineol, 2-Octanol, Trimethylpyrazine	Gu et al., 2007
Bacillus weihenstephanensis	86662	Propanone, Benzaldehyde, Phenol, Benzenacetaldehyde, Propionic acid, 1-Hexadecanol, Benzeneethanol, Phenylethanone, Cyclohexene, Nonane, 2-Undecanone, Decanal, Dodecane, Hexadecane, Dimethyldisulfide, Tetradecane, 2-Nonanone, Terpineol, 2-Octanol, Trimethylpyrazine	
Bacteroides bivius	28125	Acetic acid	Wiggins et al., 1985
Bacteroides distasonis	823	Acetic acid, Propionic acid, Isobutyric acid, Isovaleric acid	
Bacteroides fragilis	817	Acetate, Succinate, Isobutyrate, Isovalerate	Hinton and Hume, 1995
		Hexadecanoic acid, Tetradecanoic acid, 12-Methyltetradecanoic acid, 13-Methyltetradecanoic acid, 3-Hydroxy-15-methylhexadecanoic acid, 3-Hydroxyhexadecanoic acid	Brondz and Olsen, 1991
Bacteroides gracilis	824	Hexadecanoic acid, Dodecanoic acid, Octadecanoic acid, Tetradecanoic acid, 3-Hydroxytetradecanoic acid, 3-Hydroxyhexadecanoic acid, Hexadecenoic acid	
Bacteroides ovatus	28116	Acetic acid, Propionic acid, Isovaleric acid	Wiggins et al., 1985
Bacteroides thetaiotaomicron	818	Acetic acid, Propionic acid, Isobutyric acid, Isovaleric acid	
Bacteroides ureolyticus	827	Hexadecanoic acid, Dodecanoic acid, Octadecanoic acid, Tetradecanoic acid, 3-Hydroxytetradecanoic acid, 3-Hydroxyhexadecanoic acid, Hexadecenoic acid	Brondz and Olsen, 1991
Bacteroides vulgatus	821	Acetic acid, Propionic acid, Isobutyric acid, Isovaleric acid	Wiggins et al., 1985
Brevibacterium linens	1703	Methanethiol, S-Methylthiobutyrate, S-Methylthio-2-methyl butyrate, S-Methyl thioacetate, S-Methyl thiocaproate, S-Methylthio-3-methyl butyrate, S-Methyl thiopropionate, S-Methylthio-2-methyl propionate, S-Methyl thiovalerate	Schulz and Dickschat, 2007
Calothrix parietina	32054	Octanal, Decanal, 6-Methyl-5-hepten-2-one, beta-Cyclocitral, Heptadecane, Limonene, Heptadecene, Nonanal, 2,6,6-Trimethylcyclo-hex-2-en-1-one, 8-Methylheptadecane, Dihydro-beta-ionone, beta-Ionone, beta-Ionone-5,6-epoxide	Höckelmann and Jüttner, 2004
Calothrix spp.	1186	Cresol, Skatole, Sulcatone, beta-Cyclocitral, 2,2,6-Trimethylcyclohexanone, Sulcatol, Dihydroactinidiolide, 2-Hydroxy-2,6,6-trimethylcyclohexan-1-one, Dihydro-beta-ionone, beta-Ionone, (Z)-5-Heptadecene, Geosmin, beta-Ionone-5,6-epoxide	Schulz and Dickschat, 2007
		Octanal, Decanal, Heptadecane, Limonene, Nonanal, Geosmin, beta-Ionone-5,6-epoxide	Höckelmann and Jüttner, 2004
Campylobacter fetus	196	Hexadecanoic acid, Dodecanoic acid, Octadecanoic acid, Tetradecanoic acid, 3-Hydroxytetradecanoic acid, 3-Hydroxyhexadecanoic acid, Hexadecenoic acid	Brondz and Olsen, 1991
Capnocytophaga ochracea	1018	Acetic acid, Propionic acid, Isovaleric acid	Kurita-Ochiai et al., 1995
Carnobacterium divergens	2748		Ercolini et al., 2009

Table 2 (continued)

Species	Tax ID (NCBI)	Volatile Synonym	References
		Toluene, Menthol, Hexadecanol, Dibutylphthalate, Hexanal, Carbondisulfide, Isobornylacetate, Linalool, alpha-Pinene, 2-Ethylphenol, 4-Methylguaiacol, ortho-Dimethylbenzene, tert-Butylcyclohexanol, 2-Ethyl-1-hexanol, Ethyloctanoate, para-Dimethylbenzene, 4-Methylthiophenol, 1-Propanethiol, Decanal, 1-Undecanol, 1-Dodecanol, Citronellylacetate, 1-Hexadecene, Methoxybenzenethiol, 2-Nonanone, 1-Tetradecene, Decane, delta-Nonalactone, 1-Octen-3-ol, 2-Butyl-1-octanol, 2-Pentylthiophene, Limonene, 2-Ethylhexyl-2-ethyl hexanoate, 2-Dodecanol, 2-Ethylhexanal, Ethylhexanoate, Nonanal, Tetradecanal, Butylhydroxytoluene, Isotridecanol, Terbutylcyclohexyl acetate, Decenyl acetate, 2-Methyl-1-dodecanol, Hexyl formate, Ethenyl decanoate, Undecanthiol, 2-Methyl-1-undecanol, 1-Nonen-3-ol, Limoneneoxide, 9,12-Tetradecadien-1-ol acetate, 3-Hydroxydodecanoic acid, 2-Hexyl-1-decanol, 4-Methoxybenzhydrol, Tetradecen-1-ol, 5-Undecene, 5-Methyl-1,5-hexadien-3-ol, Dodecyl hexanoate, 5-Butyl-4-nonene, 2-Methyl-2-decene, 6-Methyl-1-octanol, Tetradecen-1-ol acetate, 4-Methylundecene, 8-Methyl-1-undecene, 2,3-Epoxygeranyl acetate, 2-Hexyl-1-octanol, 2-Ethyldodecanol, 2-Methyl-2-dodecene, 2-Buten-1-ol, Dodecenal, 2-Octen-1-ol, 5-Octadecene, 9-Octadecene, Tetradecenoic acid,	
Carnobacterium maltaromaticum	2751	2-Ethyl-1-hexanol, 2-Nonanone, 2-Ethylhexanal, 2-Hexyl-1-octanol, 2-Buten-1-ol	
Chondromyces crocatus	52	Benzylalcohol, 2-Phenylethanol, endo-Bornyl acetate, Benzothiazol, 1,4-Dimethoxybenzol,, 2,5-Dimethylpyrazine, 2-Methoxy-3-(2-methylpropyl)pyrazine, 2-Methoxy-3-(1-methylethyl)pyrazine, (1-Methylethyl)pyrazine, beta-Copaene, 2-Methoxy-3-(1-methylpropyl)pyrazine, alpha-Elemene, Dimethyl-(1-methylethyl)pyrazine, 2-(1-Hydroxy-2-methylpropyl)-3-methoxypyrazine, Eremophilene, 1,4-Cadinadiene, Zonarene, Bicycloelemene, 2,5-bis-(1-Methylethyl)pyrazine, 2-(1-Methylethenyl)-5-(1-methylethyl)pyrazine, 2,5-Bis(2-methylpropyl)pyrazine, (1R,6S,10S)-6,10-Dimethylbicyclo[4.4.0]decan-3-one, 2,6-bis-(1-Methylethyl)pyrazine, beta-Ylangene, Methyl salicylate, 1-Phenylethanol, Anisol, 2-Aminoacetophenone, Methyl 2-methoxy benzoate, 2-(Methoxymethyl)furan, alpha-Muurolene, (-)-Germacrene D, (6S,10S)-6,10-Dimethylbicyclo[4.4.0]dec-1-en-3-one, (1R,6R,10R)-6,10-Dimethylbicyclo[4.4.0]decan-3-one, (1(10)*E*,5*E*)-Germacradien-11-ol, Methyl anthranilate, Nonanal, 2-Methyl-3-methoxypyrazine, 2-Methyl-5-(1-methylethyl)pyrazine, Cubenol, 3-Methoxy-2,5-dimethylpyrazine, 3-Methoxy-2,6-dimethylpyrazine, 2-Methyl-6-(1-methylethyl)pyrazine, 6,10-Dimethylbicyclo[4.4.0]decan-3-ol, Geosmin	Schulz et al., 2004
		Methyl salicylate, 1-Phenylethanol, Anisol, 2-Aminoacetophenone, Methyl 2-methoxy benzoate, 2-(Methoxymethyl)furan, alpha-Muurolene, (-)-Germacrene D, (6S,10S)-6,10-Dimethylbicyclo[4.4.0]dec-1-en-3-one, (1R,6R,10R)-6,10-Dimethylbicyclo[4.4.0]decan-3-one, (1(10)E,5E)-Germacradien-11-ol, Geosmin, 4-Methoxyacetophenone, Methyl 4-methoxy benzoate, Furfuryl 3-methyl butanoate, 2-Aminobenzaldehyde, Methyl anthranilate, Nonanal, 1,4-Dimethoxybenzene, 4-Phenylbutanone, Isopropylpyrazine, 2-Methyl-5-isopropylpyrazine, alpha-Eudesmol, 2-Methoxy-3-(1-hydroxy-2-methylpropyl)pyrazine, 2,5-Diisopropylpyrazine, 2-Isopropyl-5-isopropenylpyrazine, 2-Isopropyl-5-buten-2-yl-pyrazine, Eremophilene, 2,5-Diisobutylpyrazine, (1S,6R,10R)-6,10-Dimethylbicyclo[4.4.0]decan-3-one, 2-Methyl-6-isopropylpyrazine, 2,6-Diisopropylpyrazine	Schulz and Dickschat, 2007
		4-Phenyl-2-butanone, 2-Methoxy-3-methylpyrazine, 1-epi-Cubenol, alpha-Eudesmol, 5-Methyl-2-(1-methylethyl)pyrazine, 3-Methoxy-2,5-bis(1-methylpropyl)pyrazine, Methoxydi-(1-methylethyl)pyrazine, 3-Methoxy-2-(1-methylpropyl)-5-(2-methylpropyl)pyrazine, 2-Butan-2-yl-5-propan-2-yl-pyrazine, 3-Methoxy-5-(2-methylpropyl)-2-propan-2-yl-pyrazine, 3-Methoxy-2,5-bis(2-methylpropyl)pyrazine, 5-Butan-2-yl-3-methoxy-2-propan-2-yl-pyrazine, 5-Butan-2-yl-3-methoxy-2-methyl-pyrazine, 3-Methoxy-2-methyl-5-(2-methylpropyl)pyrazine, 6-Methyl-2-(1-methylethyl)pyrazine, (1S,6S,10S)-6,10-Dimethylbicyclo[4.4.0]decan-3-one, Methyl salicylate, 1-Phenylethanol, Anisol, 2-Aminoacetophenone, Methyl 2-methoxy benzoate, 2-(Methoxymethyl)furan, alpha-Muurolene, (-)-Germacrene D, (6S,10S)-6,10-Dimethylbicyclo[4.4.0]dec-1-en-3-one, (1R,6R,10R)-6,10-Dimethylbicyclo[4.4.0]decan-3-one, (1(10)*E*,5*E*)-Germacradien-11-ol, 4-Methoxyacetophenone, Methyl 4-methoxy benzoate, Furfuryl 3-methyl butanoate, 2-Aminobenzaldehyde, Benzylalcohol, 2-Phenylethanol, endo-Bornyl acetate, Benzothiazol, 1,4-Dimethoxybenzol,, 2,5-Dimethylpyrazine, 2-Methoxy-3-(2-methylpropyl)pyrazine, 2-Methoxy-3-(1-methylethyl)pyrazine, (1-Methylethyl)pyrazine, beta-Copaene, 2-Methoxy-3-(1-methylpropyl)pyrazine, alpha-Elemene, Dimethyl-(1-methylethyl)pyrazine, 2-(1-Hydroxy-2-methylpropyl)-3-methoxypyrazine, Eremophilene, 1,4-Cadinadiene, Zonarene, Bicycloelemene, 2,5-bis-(1-Methylethyl)pyrazine, 2-(1-Methylethenyl)-5-(1-methylethyl)pyrazine, 2,5-Bis(2-methylpropyl)pyrazine, (1R,6S,10S)-6,10-Dimethylbicyclo[4.4.0]decan-3-one, 2,6-bis-(1-Methylethyl)pyrazine, beta-Ylangene, Geosmin	Dickschat et al., 2005e
		Alpha-Muurolene, Cadina-1,4-diene, Geosmin	Dickschat et al., 2005d

Table 2 (continued)

Species	Tax ID (NCBI)	Volatile Synonym	References
Citrobacter freundii	546	Methanethiol, Phenol, Dimethylselenenylsulfide	Schulz and Dickschat, 2007
Citrobacter spp.	544	2-Phenylethanol, 3-Methylbutyl propionate, 3-(Methylsulfanyl)propan-1-ol, Dimethyldisulfide, 3-Methylbutyl acetate, 2-Methylmercaptoethanol	
Clostridium bifermentans	1490	Acetic acid, Propionic acid, Isovaleric acid, Isocaproic acid	Wiggins et al., 1985
Clostridium butyricum	1492	Acetic acid, Butyric acid	
Clostridium cadaveris	1529	Acetic acid, Butyric acid, Propionic acid	
Clostridium collagenovorans	29357	Dimethylselenium, Dimethyldiselenium, Dimethyltellurium, Trimethylbismuth, Trimethylstibine, Trimethylarsine	Michalke et al., 2000
		Trimethylbismuth, Trimethylstibine, Trimethylarsine	Schulz and Dickschat, 2007
Clostridium fallax	1533	Acetic acid, Butyric acid	Wiggins et al., 1985
Clostridium histolyticum	1498	Acetic acid	
Clostridium sporogenes	1509	Acetic acid, Butyric acid, Propionic acid, Isobutyric acid, Valeric acid, Isovaleric acid, Isocaproic acid	
Clostridium spp.	1485	Acetic Acid, Acetoin, 2,3-Butanediol, Butyric acid, Formic acid, Ethanol, Methanol, Propionic Acid, Dimethylsulfide, Ethylene, Isobutanol, Acrylic acid, Isobutyric acid, Valeric acid, Caproic acid, Isovaleric acid, Isocaproic acid, Isopentanol, Crotonic acid,	Stotzky and Schenck, 1976
Clostridium tertium	1559	Acetic acid, Butyric acid	Wiggins et al., 1985
Cytophaga spp.	978	3,3,7,7-Tetramethyl-1,2,5-trithiepane, 3,3,6,6-Tetramethyl-1,2,5-trithiepane, 4,4-Dimethyltrithiolane, 4,4,6,6-Tetramethyl-1,2,5-trithiepane, 3,3,8,8-Tetramethyl-1,2,5,6-tetrathiocane, 2-Methylpropane-1,2-dithiol, 3,3,7,7-Tetramethyl-1,2,5,6-tetrathiocane, 5,5-Dimethyltetrathiane,	Sobik et al., 2007
Desulfovibrio acrylicus	41791	Methanethiol, Dimethylsulfide	Schulz and Dickschat, 2007
Desulfovibrio gigas	879	Dimethylselenium, Dimethyldiselenium, Dimethyltellurium, Trimethylarsine	Michalke et al., 2000
		Trimethylarsine	Schulz and Dickschat, 2007
Desulfovibrio vulgaris	881	Dimethylselenium, Dimethyldiselenium, Trimethylstibine, Trimethylarsine	Michalke et al., 2000
		Trimethylstibine, Trimethylarsine	Schulz and Dickschat, 2007
Dinoroseobacter shibae	215813	2-Phenylethanol, 4-Octanolide, 4-Nonanolide, 4-Undecanolide, 4-Heptanolide, Undecanal, Dodecanal, Butyl benzoate, Benzylcyanide, 1-Nonanol, 6-Methyl-5-hepten-2-one, 4-Hexanolide, 4-Decanolide, Tetramethylpyrazine, 4-Dodecanolide, S-Methylmethanethiosulfonate, 5-Nonanolide, Dimethyltrisulfide, 2-Methyl-4-pentanolide, 4-Methylquinazoline, 3-Methyl-4-pentanolide, 2-Butyl-3,6-dimethylpyrazine, 3-Butyl-2,5-dimethylpyrazine, Geranylacetone	Dickschat et al., 2005f
		S-Methylmethanethiosulfonate, S-Methylmethanethiosulfinate	Schulz and Dickschat, 2007
		2-Ethyl-5-methylpyrazine, 2-Ethyl-3,6-dimethylpyrazine, 3-Ethyl-2,5-dimethylpyrazine, 5-Methyl-2-(1-methylethyl) pyrazine, Methylmethylthiomethyldisulfide	Dickschat et al., 2005e
Dinoroseobacter spp.	309512	4-Butanolide, Octan-4-olide, Nonan-4-olide, Undecan-4-olide, Heptan-4-olide, 4-Pentanolide, Butyl benzoate, Hexan-4-olide, Decan-4-olide, Dodecan-4-olide, 2-Methylpentan-4-olide, Tetradecan-4-olide, 3-Methylpentan-4-olide	Schulz and Dickschat, 2007
Enterobacter agglomerans	549	1-(2-Pyridinyl)ethanone	
Enterobacter cloacae	550	Dimethylselenide	
Enterobacter spp.	547	Acetoin, Indole, 2-Phenylethanol, Hydroxypropanone, 3-(Methylsulfanyl)propan-1-ol, Dimethyldisulfide, 2-Methylmercaptoethanol	
Escherichia coli	562	Acetic acid, Methanol, Acetaldehyde, Acetone, 1-Butanol, Methanethiol, 2-Methyl-1-butanol, Ethanol, Indole	Bunge et al., 2008
		Acetoin, 2,3-Butanediol, 2,3-Butanedione, Glyoxylic acid, Acetylene, Isoprene, 1-Propanol-2-methyl, 2-Butanone, Diethylacetic acid, Dodecane, Ethyl acetate, 3-Methylbutanoic acid, 2-Methylfuran, Hexadecane, 3-Methylbutanal, Dimethyldisulfide, 1-Undecene, Tetrahydro-2,5-dimethylfuran, 1-Undecane, Dimethyltrisulfide, 2-Pentylfuran, 3-Methyl-1-butanol, Acetic acid butyl ester, 3-Methylbutyl acetate, 2-Hydroxy-3-pentanone, 2,4-Hexadienal, Acetone, 1-Butanol, Methanethiol, 2-Methyl-1-butanol, Ethanol	Farag et al., 2006
		Ethanol, Indole, Acetonitrile	Zhu et al., 2010

Table 2 (continued)

Species	Tax ID (NCBI)	Volatile Synonym	References
		Indole	Schulz and Dickschat, 2007; Ryan and Dow, 2008
Escherichia spp.	561	2-Phenylethanol, Ethyl octanoate, 1-Decene	Schulz and Dickschat, 2007
Flavobacterium spp.	237	Ethanol, Dimethyldisulfide	Freeman et al., 1976
Fossombronia pusilla	34161	Geosmin	Dickschat et al., 2005a
Fusobacterium nucleatum	851	Acetic acid, Butyric acid, Propionic acid, Valeric acid, Isovaleric acid	Kurita-Ochiai et al., 1995
		Methanethiol, L-Methionine	Schulz and Dickschat, 2007
Geobacillus stearothermophilus	1422	Dimethylselenide, Dimethyldiselenide, Dimethyltelluride, Dimethylditelluride, Methanetellurol	
Halomonas venusta	44935	(S)-2-Methoxy-3-(1-methylpropyl)pyrazine	
Jannaschia helgolandensis	188906	5-Methyl-2-(1-methylethyl)pyrazine	Dickschat et al., 2005e
Klebsiella oxytoca	571	Dimethyldisulfide, Dimethyltrisulfide	Schulz and Dickschat, 2007
Klebsiella pneumoniae	573	Phenol, 2-Methyl-5-isopropylpyrazine	
Klebsiella spp.	570	Acetoin, Indole, 2-Phenylethanol, 2-(Hydroxymethyl)furan, 1-Phenylpropan-2-one, Ethyl butanoate, 3-Methylbutyl butanoate, Methylpropyl acetate, 3-(Methylsulfanyl)propan-1-ol, Pentyl butanoate, Hexan-2-one, 2-Methylbutyl acetate, Dimethyldisulfide, 3-Methylbutyl acetate, 2-Methylmercaptoethanol	
Lactobacillus brevis	1580	3-Methylthiopropionate, Methanethiol, 3-(Methylsulfanyl)propan-1-ol, Dimethyldisulfide, Methional	
Lactobacillus casei	1582	Acetic acid, Acetoin, Benzaldehyde, Benzenemethanol, Butanoic acid, Octanoic acid, Decanoic acid, Dodecanoic acid, 2-Phenylethanol, Isobutanol, Ethyl-2-hydroxy propionate, Pentanoic acid, Heptanoic acid, Nonanoic acid, Hexanoic acid, 3-(Methylthio)-1-propanol, Tetradecanoic acid, 3-Methyl-2-butanol, alpha,alpha-Dimethylbenzenemethanol, Isoamylalcohol	Tracey and Britz, 1989
Lactobacillus fermentum	1613	L-Cystathionine	Schulz and Dickschat, 2007
Lactobacillus hilgardii	1588	3-Methylthiopropionate, Methanethiol, 3-(Methylsulfanyl)propan-1-ol, Dimethyldisulfide	
Lactobacillus lactis	29397	alpha-keto-gamma-methylthiobutyric acid, Methanethiol, Methional, Methylmercaptoacetaldehyde	
Lactobacillus plantarum	1590	Acetic acid, Acetoin, Benzenemethanol, Butanoic acid, Octanoic acid, Decanoic acid, Dodecanoic acid, 2-Phenylethanol, Isobutanol, Ethyl-2-hydroxy propionate, Pentanoic acid, Heptanoic acid, Hexanoic acid, 3-(Methylthio)-1-propanol, Tetradecanoic acid, 3-Methyl-2-butanol, alpha,alpha-Dimethylbenzenemethanol, Isoamylalcohol, Benzaldehyde	Tracey and Britz, 1989
		3-Methylthiopropionate, Methanethiol, Phenylpyruvate, L-Phenylalanine, 3-(Methylsulfanyl)propan-1-ol, Dimethyldisulfide, Benzaldehyde	Schulz and Dickschat, 2007
Lactobacillus spp.	1578	Methanethiol, Dimethylsulfide, Dimethyldisulfide, Dimethyltrisulfide	
Lactococcus lactis	1358	Acetic acid, Acetoin, Benzaldehyde, Benzenemethanol, Butanoic acid, Octanoic acid, Decanoic acid, Dodecanoic acid, 2-Phenylethanol, Isobutanol, Ethyl-2-hydroxy propionate, Pentanoic acid, Heptanoic acid, Nonanoic acid, Hexanoic acid, 3-(Methylthio)-1-propanol, Tetradecanoic acid, 3-Methyl-2-butanol, alpha,alpha-Dimethylbenzenemethanol, Isoamylalcohol	Tracey and Britz, 1989
		L-Cystathionine, S-Methyl thiobutyrate, S-Methylthio-2-methyl butyrate, S-Methyl thioacetate, S-Methyl thiocaproate, S-Methyl thio-3-methyl butyrate, S-Methyl thiopropionate, S-Methylthio-2-methyl propionate, S-Methyl thiovalerate	Schulz and Dickschat, 2007
Lactococcus spp.	1357	Methanethiol, Phenyl pyruvate, Phenylacetaldehyde, Dimethylsulfide, L-Phenylalanine, Dimethyldisulfide, Dimethyltrisulfide	
Leuconostoc cremoris	33965	Acetic acid, Acetoin, Benzaldehyde, Benzenemethanol, Butanoic acid, Octanoic acid, Decanoic acid, Dodecanoic acid, 2-Phenylethanol, Isobutanol, Ethyl-2-hydroxy propionate, Pentanoic acid, Heptanoic acid, Nonanoic acid, Hexanoic acid, 3-(Methylthio)-1-propanol, Tetradecanoic acid, 3-Methyl-2-butanol, alpha,alpha-Dimethylbenzenemethanol, Isoamylalcohol	Tracey and Britz, 1989
Leuconostoc dextranicum	33966	Acetic acid, Acetoin, Benzaldehyde, Benzenemethanol, Butanoic acid, Octanoic acid, Decanoic acid, Dodecanoic acid, 2-Phenylethanol, Isobutanol, Ethyl-2-hydroxy propionate, Pentanoic acid, Heptanoic acid, Nonanoic acid, Hexanoic acid, 3-(Methylthio)-1-propanol, Tetradecanoic acid, 3-Methyl-2-butanol, alpha,alpha-Dimethylbenzenemethanol, Isoamylalcohol	

Table 2 (continued)

Species	Tax ID (NCBI)	Volatile Synonym	References
Leuconostoc mesenteroides	1245	Acetoin, Benzaldehyde, Benzenemethanol, Butanoic acid, Octanoic acid, Decanoic acid, Dodecanoic acid, 2-Phenylethanol, Isobutanol, Ethyl-2-hydroxy propionate, Pentanoic acid, Heptanoic acid, Nonanoic acid, Hexanoic acid, 3-(Methylthio)-1-propanol, Tetradecanoic acid, 3-Methyl-2-butanol, alpha,alpha-Dimethylbenzenemethanol, Isoamylalcohol	
Leuconostoc oenos	1247	Acetic acid, Acetoin, Benzaldehyde, Benzenemethanol, Butanoic acid, Octanoic acid, Decanoic acid, Dodecanoic acid, 2-Phenylethanol, Isobutanol, Ethyl-2-hydroxy propionate, Pentanoic acid, Heptanoic acid, Nonanoic acid, Hexanoic acid, 3-(Methylthio)-1-propanol, Tetradecanoic acid, 3-Methyl-2-butanol, alpha,alpha-Dimethylbenzenemethanol, Isoamylalcohol	
Leuconostoc paramesenteroides	1249	Acetoin, Benzaldehyde, Benzenemethanol, Butanoic acid, Octanoic acid, Decanoic acid, Dodecanoic acid, 2-Phenylethanol, Isobutanol, Pentanoic acid, Heptanoic acid, Nonanoic acid, Hexanoic acid, 3-(Methylthio)-1-propanol, Tetradecanoic acid, 3-Methyl-2-butanol, alpha,alpha-Dimethylbenzenemethanol, Isoamylalcohol	
Loktanella hongkongensis	278132	Tetramethylpyrazine, 2-Ethyl-5-methylpyrazine, 3-Ethyl-2,5-dimethylpyrazine, 5-Methyl-2-(1-methylethyl)pyrazine, 2,5-Dimethyl-3-(3-methylbutyl)pyrazine, 3-Butyl-2,5-dimethylpyrazine	Dickschat et al., 2005e
Loktanella spp.	245186	Indole, 4-Butanolide, Octan-4-olide, Nonan-4-olide, Undecan-4-olide, Heptan-4-olide, 4-Pentanolide, S-Methyl methanethiosulfonate, 4-(Methylsulfanyl)butan-2-one, S-Methylmethanethiosulfinate, Tetradecan-4-olide, Tropone, Methyl 2-furancarboxylate, 1-Phenylpropan-1,2-dione, Hexan-4-olide, Decan-4-olide, Dodecan-4-olide, 3-Methylbutan-4-olide, S-Methyl thiopropionate	Schulz and Dickschat, 2007
		Indole, 2-Phenylethanol, 4-Nonanolide, 4-Pentanolide, Undecanal, 1-Tetradecanol, Benzycyanide, 6-Methyl-5-hepten-2-one, Dimethyltrisulfide, 2-Ethyl-3,6-dimethylpyrazine, 4-Methylthio-2-butanone, Methylmethylthiomethyldisulfide, 4-Methylquinazoline, 2-Isopentyl-3,6-dimethylpyrazine, 2-Butyl-3,6-dimethylpyrazine, Tetramethylpyrazine, Geranylacetone, 1-Phenylpropan-1,2-dione, Hexan-4-olide, Decan-4-olide, Dodecan-4-olide, 3-Methylbutan-4-olide, S-Methyl thiopropionate	Dickschat et al., 2005f
Lyngbya spp.	28073	Geosmin	Schulz and Dickschat, 2007
Lysobacter gummosus	262324	Acetamide, Benzaldehyde, Phenylacetaldehyde, Methanamine, Benzothiazole, Methylpyrazine, 1-Butanamine, Dodecane, Dimethyldisulfide, Nonadecane, 1-Decene, 2,5-Dimethylpyrazine	Zou et al., 2007
Methanobacterium formicicum	2162	Stibine, Dimethylselenium, Trimethylbismuth, Trimethylstibine, Dimethyldiselenium, Dimethyltellurium, Trimethylarsines, Dimethylstibine, Monomethylstibine, Monomethylarsine, Dimethylarsine	Michalke et al., 2000
		Trimethylbismuth, Monomethylarsine, Dimethylarsine	Schulz and Dickschat, 2007
Methanobacterium spp.	2160	Trimethylarsine, Dimethylarsine	Stotzky and Schenck, 1976
Methanobacterium thermoautotrophicum	145262	Trimethylstibine	Michalke et al., 2000
Methanosarcina barkeri	2208	Dimethylselenium, Trimethylstibine, Dimethyldiselenium	
Methylobacterium spp.	407	Methyliodide	Schulz and Dickschat, 2007
Microbacterium oxydans	82380	Propanone, Benzaldehyde, Phenol, Benzenacetaldehyde, Propionic acid, 1-Hexadecanol, Benzeneethanol, Phenylethanone, Cyclohexene, Nonane, 2-Undecanone, Decanal, Dodecane, Hexadecane, Dimethyldisulfide, Tetradecane, 2-Nonanone, Terpineol, 2-Octanol, Trimethylpyrazine	Gu et al., 2007
Microbacterium thermosphactum	2756	Ethanol, Methanol	Freeman et al., 1976
Myxococcus spp.	32	5-Methylhexan-3-ol, 7-Methyloctan-3-one, 5-Methyl-4-hexen-3-one	Schulz and Dickschat, 2007
Myxococcus xanthus	34	2-Phenylethanol, Benzothiazole, Benzylcyanide, 6-Methyl-5-hepten-2-one, Butyl propionate, 5-Methylhexan-3-ol, 5-Methylhexan-3-one, Tridecane, 2-Acetylfuran, Dimethyltrisulfide, Butyl acetate, 7-Methyloctan-3-one, 5-Methylhex-4-en-3-one, Cyanoisoquinoline, (3S)-Decan-3-ol, 4-Methylquinoline, 2-Aminoacetophenone, Decan-3-one, Nonan-3-one, Undecan-3-one, Dimethyltetrasulfide, Octan-3-one, Geranylacetone, Geosmin, (-)-Germacrene D, 9-Methyldecan-3-one, (1(10)E,5E)-Germacradien-11-ol	Dickschat et al., 2004
		Sulcatone, Undecan-3-ol, (S)-Decan-3-ol, Isolepidozene, Octalinhydrocarbon, 4-Methylquinoline, 2-Aminoacetophenone, Decan-3-one, Nonan-3-one, Undecan-3-one, Dimethyltetrasulfide, Octan-3-one, Geranylacetone, Geosmin, (-)-Germacrene D, 9-Methyldecan-3-one, (1(10)E,5E)-Germacradien-11-ol	Schulz and Dickschat, 2007

Table 2 (continued)

Species	Tax ID (NCBI)	Volatile Synonym	References
		Geosmin	Dickschat et al., 2005d
		(-)-Germacrene D, 9-Methyldecan-3-one, (1(10)E,5E)-Germacradien-11-ol	Dickschat et al., 2005a
		8,10-Dimethyl-1-octalin, (1(10)E,5E)-Germacradien-11-ol, Geosmin	Nawrath et al., 2008
Nannocystis exedens	54	Benzylalcohol, Hexadecan-1-ol, 2-Phenylethanol, Isobornyl acetate, Benzothiazole, Ethyl 2-methyl propionate, Heptan-4-olide, 2-Butyl acetate, 4-Pentanolide, Ethyl-3-methyl butyrate, Dodecan-1-ol, Tetradecan-1-ol, Benzylnitrile, Pinanol, 2-Aminoacetophenone, Methyl-2-furan carboxylate, Hexan-4-olide, (-)-2-Methylisoborneol, Limonene, Ethyl-2-methyl butyrate, Diethyl succinate, Borneol, 1-Phenyldecan-1-one, 2-Methylenebornane, 2-Methyl-2-bornene, 2,5-Dimethyl-3-(1-methylethyl)pyrazine, Germacrene D, (6S,10S)-6,10-Dimethylbicyclo[4.4.0]dec-1-en-3-one, 2,5-Di-(1-methylethyl)pyrazine, 2-(1-Methylethenyl)-5-(1-methylethyl)pyrazine, 2,5-Di-(1-methylpropyl)pyrazine, 5-(1-Methylethyl)-2-(1-methylpropyl)pyrazine, (1(10)E,5E)-Germacradien-11-ol, beta-Ylangene, Geosmin	Dickschat et al., 2007
		2-Furanmethanol, Geosmin	Schulz and Dickschat, 2007
		Geosmin	Schulz et al., 2004; Dickschat et al.,2005a, d
		8,10-Dimethyl-1-octalin	Nawrath et al., 2008
Nannocystis exedens subsp. *cinnabarina*	54	2-Phenylethanol	Dickschat et al., 2007
Oceanibulbus indolifex	225422	2-Ethyl-5-methylpyrazine, 3-Ethyl-2,5-dimethylpyrazine, 5-Methyl-2-(1-methylethyl)pyrazine	Dickschat et al., 2005e
Octadecabacter spp.	53945	Benzaldehyde, 2-Acetylfuran, 2,5-Dimethylpyrazine, 4-(Methylsulfanyl)butan-2-one	Dickschat et al., 2005a
		4-(Methylsulfanyl)butan-2-one, (R)-4-(Methylsulfanyl)butan-2-ol	Schulz and Dickschat, 2007
Oenococcus oeni	1247	3-Methylthiopropionate, Methanethiol, 3-(Methylsulfanyl)propan-1-ol, Dimethyldisulfide, Methional	
Oscillatoria chalybea	41313	2-Methylisoborneol	
Oscillatoria spp.	1158	2-Methylisoborneol, Geosmin	
Paenibacillus polymyxa	1406	n-Hexadecanoic acid, Octadecanoic acid, Diethylphthalate, Hexadecanoic acid methyl ester, Octadecanoic acid methyl ester, Azulene, Di-2-propenyltrisulfide,, Diallyldisulfide, Tetradecanal, 1,3-Dithiole-2-thione, Oleic acid, (Z)-9-Hexadecenoic acid methyl ester, 2,4-Decadienal, 2-Undecenal	Wei-wei et al., 2008
		Isopropylpyrazine, 2-(2-Methylpropyl)pyrazine, 2-Methyl-5-isopropylpyrazine, 2,6-Diisobutylpyrazine, 2,5-Diisopropylpyrazine, 2-Isopropyl-5-buten-2-yl-pyrazine, 2,5-Diisobutylpyrazine, 2-Methyl-5-isobutylpyrazine, 2-Methyl-6-isopropylpyrazine, 2,6-Diisopropylpyrazine	Schulz and Dickschat, 2007
		2,5-Dimethyl-3-(2-methylpropyl)pyrazine	Dickschat et al., 2005e
Parasporobacterium paucivorans	115544	Methanethiol, Dimethylsulfide	Schulz and Dickschat, 2007
Pediococcus damnosus	51663	Acetic acid, Acetoin, Benzaldehyde, Benzenemethanol, Butanoic acid, Octanoic acid, Decanoic acid, Dodecanoic acid, 2-Phenylethanol, Isobutanol, Ethyl-2-hydroxy propionate, Pentanoic acid, Heptanoic acid, Nonanoic acid, Hexanoic acid, 3-(Methylthio)-1-propanol, Tetradecanoic acid, 3-Methyl-2-butanol, alpha,alpha-Dimethylbenzenemethanol, Isoamylalcohol	Tracey and Britz, 1989
Phormidium spp.	1198	Octanal, alpha-Pinene, 2-Heptanone, Decanal, 6-Methyl-5-hepten-2-one, beta-Cyclocitral, 2-Tridecanone, Heptadecane, 2-Decanone, 6-Methylheptan-2-one, Limonene, Heptadecene, 7-Methylheptadecane, Nonanal, 1-Octen-3-one, 2,6,6-Trimethylcyclo-hex-2-en-1-one, 8-Methylheptadecane, 2-Decenal, Geosmin, Dihydro-beta-ionone, beta-Ionone, beta-Ionone-5,6-epoxide	Höckelmann et al., 2004
		Sulcatone, Dihydroactinidiolide, 2-Hydroxy-2,6,6-trimethylcyclohexan-1-one, Tetrahydroionone, Dihydro-beta-ionol, 4-Oxodihydro-beta-ionone, Geosmin, Dihydro-beta-ionone, beta-Ionone, beta-Ionone-5,6-epoxide	Schulz and Dickschat, 2007
Photobacterium spp.	657	Methyl iodide	
Plantibacter spp.	190323	Methyl iodide	
Plectonema spp.	1183		

Table 2 (continued)

Species	Tax ID (NCBI)	Volatile Synonym	References
		Octanal, Decanal, 6-Methyl-5-hepten-2-one, Heptadecane, Limonene, 7-Methylheptadecane, Nonanal, 2,6,6-Trimethylcyclo-hex-2-en-1-one, 8-Methylheptadecane, Beta-Cyclocitral, Dihydro-beta-ionone, beta-Ionone, beta-Ionone-5,6-epoxide	Höckelmann and Jüttner, 2004
		Sulcatone, Beta-Cyclocitral, Dihydro-beta-ionone, beta-Ionone, beta-Ionone-5,6-epoxide, 2,2,6-Trimethylcyclohexanone, Sulcatol, Dihydroactinidiolide, 2-Hydroxy-2,6,6-trimethylcyclohexan-1-one, beta-Cyclogeraniol, Dihydro-beta-ionol	Schulz and Dickschat, 2007
Porphyromonas endodontalis	28124	Hexadecanoic acid, Tetradecanoic acid, 12-Methyltetradecanoic acid, 11-Methyldodecanoic acid, 13-Methyltetradecanoic acid, 3-Hydroxy-15-Methylhexadecanoic acid, 3-Hydroxypentadecanoic acid, 3-Hydroxyhexadecanoic acid	Brondz and Olsen, 1991
Porphyromonas gingivalis	837	Acetic acid, Butyric acid, Propionic acid, Isobutyric acid, Valeric acid, Isovaleric acid	Kurita-Ochiai et al., 1995
		Methanethiol	Schulz and Dickschat, 2007
Prevotella buccae	28126	Hexadecanoic acid, Tetradecanoic acid, 12-Methyltridecanoic acid, 12-Methyltetradecanoic acid, 14-Methylhexadecanoic acid, 11-Methyldodecanoic acid, 14-Methylpentadecanoic acid, 13-Methyltetradecanoic acid, 3-Hydroxy-15-Methylhexadecanoic acid, 15-Methylhexadecanoic acid, 3-Hydroxypentadecanoic acid, 3-Hydroxyhexadecanoic acid, 10-Methyldodecanoic acid	Brondz and Olsen, 1991
Prevotella disiens	28130	Hexadecanoic acid, Tetradecanoic acid, 12-Methyltridecanoic acid, 12-Methyltetradecanoic acid, 14-Methylhexadecanoic acid, 11-Methyldodecanoic acid, 14-Methylpentadecanoic acid, 13-Methyltetradecanoic acid, 3-Hydroxy-15-Methylhexadecanoic acid, 15-Methylhexadecanoic acid, 3-Hydroxypentadecanoic acid, 3-Hydroxyhexadecanoic acid, 10-Methyldodecanoic acid	
Prevotella heparinolyticus	28113	Hexadecanoic acid, Tetradecanoic acid, 12-Methyltetradecanoic acid, 11-Methyldodecanoic acid, 13-Methyltetradecanoic acid, 3-Hydroxy-15-Methylhexadecanoic acid, 15-Methylhexadecanoic acid, 3-Hydroxypentadecanoic acid, 3-Hydroxyhexadecanoic acid, 10-Methyldodecanoic acid	
Prevotella intermedia	28131	Acetic acid, Butyric acid, Isobutyric acid, Isovaleric acid	Kurita-Ochiai et al., 1995
Prevotella loescheii	840	Acetic acid, Butyric acid, Propionic acid, Isobutyric acid, Valeric acid, Isovaleric acid	
Prevotella oralis	28134	Hexadecanoic acid, Tetradecanoic acid, 12-Methyltridecanoic acid, 12-Methyltetradecanoic acid, 14-Methylhexadecanoic acid, 11-Methyldodecanoic acid, 14-Methylpentadecanoic acid, 13-Methyltetradecanoic acid, 3-Hydroxy-15-Methylhexadecanoic acid, 15-Methylhexadecanoic acid, 3-Hydroxypentadecanoic acid, 3-Hydroxyhexadecanoic acid	Brondz and Olsen, 1991
Prevotella oris	28135	Hexadecanoic acid, Tetradecanoic acid, 12-Methyltridecanoic acid, 12-Methyltetradecanoic acid, 14-Methylhexadecanoic acid, 11-Methyldodecanoic acid, 14-Methylpentadecanoic acid, 13-Methyltetradecanoic acid, 3-Hydroxy-15-Methylhexadecanoic acid, 15-Methylhexadecanoic acid, 3-Hydroxypentadecanoic acid, 3-Hydroxyhexadecanoic acid, 10-Methyldodecanoic acid	
Prevotella spp.	838	11-Methyldodecanoic acid, 13-Methyltetradecanoic acid	
Prevotella veroralis	28137	Hexadecanoic acid, Tetradecanoic acid, 12-Methyltridecanoic acid, 12-Methyltetradecanoic acid, 11-Methyldodecanoic acid, 14-Methylpentadecanoic acid, 13-Methyltetradecanoic acid, 3-Hydroxy-15-Methylhexadecanoic acid, 3-Hydroxyhexadecanoic acid, 10-Methyldodecanoic acid	
Pseudoalteromonas spp.	53246	Methyliodide	Schulz and Dickschat, 2007
Pseudomonas aeruginosa	287	Ethyleneglycol, Acetic acid, Acetone, Ethanol, Indole, 4-Methylphenol, Acetonitrile, 2-Pentanone, 2-Aminoacetophenone	Zhu et al., 2010
		Butanol, 2-Undecanone, Dimethyldisulfide, 2-Nonanone, Dimethyltrisulfide, Isopentanol, Undecene, 2-Aminoacetophenone	Labows et al., 1980
Pseudomonas aurantiaca	86192	Benzaldehyde, Benzothiazole, 2-Ethyl-1-hexanol, Phenylenediamine, Cyclohexanol, 2-Methylpyrazine, Nonane, 2-Undecanone, Decanol, Dodecane, Pyrazine, 2-Tridecanone, Tetradecane, Pentadecane, Nonadecane, 1-Undecene, Undecane, 1-Heptadecanol, Decane, 4-Octylbenzoic acid, Dimethyltrisulfide, Nonanal, Hexadecane	Fernando et al., 2005
Pseudomonas cepacia	292	Dimethyldisulfide, Dimethyltrisulfide	Labows et al., 1980
Pseudomonas chlororaphis	587753		Fernando et al., 2005

Table 2 (continued)

Species	Tax ID (NCBI)	Volatile Synonym	References
		Benzaldehyde, Benzothiazole, 2-Ethyl-1-hexanol, Phenylenediamine, Cyclohexanol, 2-Methylpyrazine, Nonane, 2-Undecanone, Decanol, Dodecane, Pyrazine, 2-Tridecanone, Tetradecane, Pentadecane, Nonadecane, 1-Undecene, Undecane, 1-Heptadecanol, Decane, 4-Octylbenzoic acid, Dimethyltrisulfide, Nonanal, Hexadecane	
		Benzothiazole	Schulz and Dickschat, 2007
Pseudomonas corrugata	47879	Benzaldehyde, Benzothiazole, 2-Ethyl-1-hexanol, Phenylenediamine, Cyclohexanol, 2-Methylpyrazine, Nonane, 2-Undecanone, Decanol, Dodecane, Pyrazine, 2-Tridecanone, Tetradecane, Pentadecane, Nonadecane, 1-Undecene, Undecane, 1-Heptadecanol, Decane, 4-Octylbenzoic acid, Dimethyltrisulfide, Nonanal, Hexadecane	Fernando et al., 2005
Pseudomonas doudoroffii	84158	Methanethiol, Dimethylsulfide	Schulz and Dickschat, 2007
Pseudomonas fluorescens	294	Acetoin	Lee et al., 1979; Pittard et al.,1982
		Benzaldehyde	Lee et al., 1979; Fernando et al., 2005
		2,3-Butanediol, 1-Butanol, 2,3-Butanedione, Glyoxylic acid, 1-Pentanol, Acetylene, Isoprene, 2-Methyl-1-propanol, Diethylacetic acid, 2-Methylbutanal, Cyclohexane, 2-Methyl-1-butanol, 2-Methylfuran, Hexadecane, Tetrahydro-2,5-dimethylfuran, 3-Methyl-1-butanol, Butanol-3-methyl acetate, 2-Hydroxy-3-pentanone, 2,4-Hexadienal, Benzaldehyde, Acetoin, Ethanol, Methanethiol, 2-Butanone, Dodecane, Undecane, Methylbutanal, Dimethyldisulfide, 1-Undecene, Dimethyltrisulfide	Farag et al., 2006
		Butanol, Isopentanol, Dimethyltrisulfide	Labows et al., 1980
		Ethanol, Methanol, Methyl propionate, Dimethylsulfide, Dimethyldisulfide, Methylthiol acetate	Freeman et al., 1976
		Methanethiol, 2-Butanone, Dimethylsulfide, Toluene, 2-Nonanone, 4-Methyl-2,6-di-tert-butylphenol, Dimethyldisulfide, 1-Undecene, Dimethyltrisulfide, Nonanal, Methylthiol acetate, 2-Butanol, 3-Octanone, Dimethylbenzenes, Ethylmethyldisulfide, 2-Octanol, 1-Nonene, Cycloheptene, 4-Octanone, 2-Pentanone, 2-Heptanone, Trimethylbenzene, 3-Pentanone	Pittard et al., 1982
		Toluene, 2-Nonanone, 4-Methyl-2,6-di-tert-butylphenol, Methyl propionate, Methyl isothiocyanate, Methyl-2-methyl butyrate, Methylpent-2-enoate, Methylbutanal, Dimethyldisulfide, 1-Undecene, Dimethyltrisulfide, Methylthiol acetate	Lee et al., 1979
		Benzothiazole, 2-Ethyl-1-hexanol, Phenylenediamine, Cyclohexanol, 2-Methylpyrazine, Nonane, 2-Undecanone, Decanol, Tetradecane, Pentadecane, Nonadecane, 1-Heptadecanol, Decane, 4-Octylbenzoic acid, Hexadecane, 2-Tridecanone, Pyrazine, Dodecane, Undecane, 1-Undecene, Dimethyltrisulfide, Nonanal	Fernando et al., 2005
		Dimethyltelluride	Schulz and Dickschat, 2007
		Undecene	Kai et al., 2007
Pseudomonas fragi	296	Acetaldehyde, Ethylalcohol, Methylmercaptan, Butanone, Ethyl butyrate, Ethyl hexanoate, Dimethylsulfide, Ethyl acetate, Dimethyldisulfide	Miller et al., 1973
		Ethanol, Methanol, Methyl acetate, Dimethylsulfide, Ethyl acetate, Dimethyldisulfide	Freeman et al., 1976
		Toluene, Menthol, Dibutylphthalate, Hexanal, Carbondisulfide, Linalool, alpha-Pinene, 2-Ethylphenol, 4-Methylguaiacol, 2-Ethyl-1,3-hexandiol, ortho-Dimethylbenzene, para-Dimethylbenzene, 1-Butene, 2,4,4-Trimethyl-1-pentene, Decanal, Dodecene, 10-Undecenal, 1-Dodecanol, 4(1,1,3,3-Tetramethylbutyl)phenol, 1-Nonanol, Citronellyl acetate, 1-Hexadecene, 2-Undecanol, alpha-Terpineol, 1-Octen-3-ol, 2-Butyl-1-octanol, 1,9-Nonanediol, 2-Pentylthiophene, Limonene, 2-Ethylhexyl-2-ethyl hexanoate, 2-Dodecanol, Tridecanal, Nonanal, Butylhydroxytoluene, Decenyl acetate, 2-Methyl-1-dodecanol, Hexadecanediol, 2-Methyl-1-decanol, 1-Nonen-3-ol, 1,2-Dodecanediol, 3-Hydroxydodecanoic acid, 2-Methyl-3-buten-1-ol, 2-Hexyl-1-decanol, 4-Methoxybenzhydrol, 2-Pentadecanol, Tetradecen-1-ol, Dodecyl hexanoate, 5-Butyl-4-nonene, 2-Methyl-2-decene, 5-Methylundecene, 2,3-Epoxygeranyl acetate, 2-Ethyldodecanol, 2-Ethyl-1-decanol, 2-Methyl-2-dodecene, Dodecenal, 3-Tetradecene, 3-Decen-2-one, Undecene, 6-Dodecenol, 2,5-Octanedione, 2-Methylundecanthiol, 2-Butyloctenal	Ercolini et al., 2009
Pseudomonas maltophilia	40324	Butanol, 2-Undecanone, Dimethyldisulfide, Dimethyltrisulfide, Isopentanol	Labows et al., 1980
Pseudomonas putida	303		Lee et al., 1979

Table 2 (continued)

Species	Tax ID (NCBI)	Volatile Synonym	References
		Acetone, 4-Methyl-2,6-di-tert-butylphenol, Methylthiol acetate, 2-Nonanone, 2-Butanone, Toluene, Benzaldehyde, Methyl benzoate, Methyl isothiocyanate, Methyl butanal, Methylpent-2-enoate, 1-Undecene, Dimethyldisulfide, Dimethyltrisulfide	
		Butanol, Isopentanol, 2-Undecanone, Dimethyldisulfide, Dimethyltrisulfide	Labows et al., 1980
		Ethanol, Heptadiene, Methanol, Dimethyldisulfide	Freeman et al., 1976
		Methanethiol, 3-Pentanone, 2-Pentanone, 2-Heptanone, Trimethylbenzene, 4-Octanone, n-Nonanal, Ethylmethyldisulfide, 3-Octanone, Dimethylbenzenes, Acetone, 4-Methyl-2,6-di-tert-butylphenol, Methylthiol acetate, 2-Nonanone, 2-Butanone, Toluene, Carbondisulfide, Dimethyldisulfide, Dimethyltrisulfide	Pittard et al., 1982
		Phenylacetate, Carbondisulfide	Schulz and Dickschat, 2007
Pseudomonas putrefaciens	24	Butanol, Dimethyltrisulfide, Isopentanol, Dimethyldisulfide	Labows et al., 1980
		Ethanol, Methylmercaptan, Methanol, Dimethyldisulfide	Freeman et al., 1976
Pseudomonas solanacearum	305	Ethylene	Stotzky and Schenk, 1976
Pseudomonas spp.	286	Methylmercaptan, Methyl acetate, Ethylbenzene, Ethyl acetate, Dimethyldisulfide, Xylene, Dimethylsulfide	Freeman et al., 1976
		Methyliodide, 1-Undecene, Dimethylselenenylsulfide	Schulz and Dickschat, 2007
		Methylthiol n-butyrate	Pittard et al., 1982
		6-Methyl-1-octanol, Tetradecen-1-ol acetate, 4-Methylundecene	Ercolini et al., 2009
Pseudomonas taetrolens	47884	2-Methoxy-3-(1-methylethyl)pyrazine	Schulz and Dickschat, 2007
Pseudomonas trivialis	200450	Undecadiene, Benzyloxybenzonitrile, Undecene	Kai et al., 2007
Pseudonocardia spp.	1847	Isoprene	Schulz and Dickschat, 2007
Rhizobium spp.	379	Methyliodide	
Rhodobacter sphaeroides	1063	Dimethylselenide	
Rhodococcus spp.	1827	Methyliodide	
Rhodocyclus tenuis	1066	Dimethylselenide, Dimethyltelluride	
Rhodospirillum rubrum	1085		
Rivularia spp.	373984	2-Heptanone, 6-Methyl-5-hepten-2-one, beta-Cyclocitral, 2-Tridecanone, 2-Decanone, Limonene, 2,6,6-Trimethylcyclohex-2-en-1-one, 8-Methylheptadecane, beta-Ionone-5,6-epoxide, Geosmin	Höckelmann et al., 2004
		Sulcatone, Geosmin	Schulz and Dickschat, 2007
Roseobacter gallaeciensis	60890	2-Ethyl-5-methylpyrazine, 3-Ethyl-2,5-dimethylpyrazine, 5-Methyl-2-(1-methylethyl)pyrazine	Dickschat et al., 2005a
Roseobacter spp.	2433	Methanethiol, Dimethylsulfide, 2-Phenylethanol	Schulz and Dickschat, 2007
Roseovarius spp.	74030	Methyliodide, Diiodomethane, Triiodomethane, Chloroiodomethane	
Saccharomonospora spp.	1851	Isoprene	
Salmonella enterica	28901	Acetic acid, Acetaldehyde, 1-Butanol, Ethanol, Methanethiol, Methanol, 2-Butanone, 2-Methyl-1-butanol	Bunge et al., 2008
Salmonella enterica serovar typhimurium	90371	Ethyleneglycol, Acetic acid, Acetone, Butanol, Ethanol, Indole, 4-Methylphenol, Acetonitrile, 2-Pentanone, Pyrimidine, 2-Nonanone, Isopentanol	Zhu et al., 2010
Serratia marcescens	615	Propanone, Benzaldehyde, Phenol, Benzenacetaldehyde, Propionic acid, 1-Hexadecanol, Benzeneethanol, Phenylethanone, Cyclohexene, Nonane, 2-Undecanone, Decanal, Dodecane, Hexadecane, Dimethyldisulfide, Tetradecane, 2-Nonanone, Terpineol, 2-Octanol, Trimethylpyrazine	Gu et al., 2007
Serratia odorifera 4Rx13	618	Beta-Phenylethanol, Dimethyldisulfide, Dimethyltrisulfide, Methantiol, ‚Sodorifen’	Kai et al., 2007 and 2010
Serratia plymuthica HROC48	82996	Beta-Phenylethanol, Benzylnitrile, trans-9-Hexadecene-1-ol	Kai et al., 2007
Serratia proteamaculans	28151	Toluene, Menthol, Hexanal, Carbondisulfide, Linalool, alpha-Pinene, 2-Ethylphenol, 4-Methylguaiacol, ortho-Dimethylbenzene, Ethyl octanoate, para-Dimethylbenzene, 1-Propanethiol, Ethyl decanoate, Citronellyl acetate, 1-	Ercolini et al., 2009

Table 2 (continued)

Species	Tax ID (NCBI)	Volatile Synonym	References
		Methyl-2,1-methylethylbenzene, Methylethylsulfide, 1-Hexadecene, 2-Hexen-1-ol, alpha-Terpineol, 1-Octen-3-ol, 2-Pentylthiophene, Limonene, 2-Ethylhexyl-2-ethyl hexanoate, 2-Dodecanol, Ethyl nonanoate, 3-Methyl-1-butanol, Ethyl hexanoate, Isoamyl acetate, Butylhydroxytoluene, Decenyl acetate, 2-Hexen-1-ol propanoate, Linalyl propanoate, Borneol, 1-Nonen-3-ol, 3-Hydroxydodecanoic acid, 4-Methoxybenzhydrol, Tetradecen-1-ol, Dodecyl hexanoate, 3-Octanone, 5-Butyl-4-nonene, 6-Methyl-1-octanol, Tetradecen-1-ol acetate, 4-Methylundecene, 2,3-Epoxygeranyl acetate, 4-Hydroxy-3-methylbutanal, Dodecenal, trans-2-Hexen-1-ol, 2-Octen-1-ol, 2-Nonen-1-ol	
Serratia spp.	613	Acetic acid, 3-Hydroxy-2-butanone, 2-Propanone, Ethanol, 1,2-Benzenedicarboxylic acid, 2-Propanol, 2-Methyl-1-propanol, 2-Butanone, 2-Methyl-propanoic acid, 1,2-Dimethylbenzene, 2-Ethyl-1-hexanol, 2-Pentanone, Acetic acid ethenyl ester, 2-Heptanone, 2-Undecanone, 2-Methyl-butanoic acid, Ethyl acetate, 3-Methyl-butanoic acid, 2-Nonanone, Undecane, 2-Dodecanone, 2,5-Dimethylpyrazine, Limonene, Dimethyldisulfide, Dimethyltrisulfide	Bruce et al., 2004
		Acetoin, Undecan-2-one, Nonan-2-one, Dodecan-2-one, Dimethyldisulfide, Dimethyltrisulfide	Schulz and Dickschat, 2007
Shewanella spp.	22	Methyliodide, 1-Undecene	
Shigella flexneri	623	Acetic acid, Acetaldehyde, Acetone, 1-Butanol, Ethanol, Indole, Methanethiol, Methanol, 2-Methyl-1-butanol	Bunge et al., 2008
Sphingomonas spp.	13687	Methyliodide	Schulz and Dickschat, 2007
Spirulina platensis	118562	beta-Ionone-5,6-epoxide	
Sporosarcina ginsengisoli	363855	Acetamide, Benzaldehyde, Phenylacetaldehyde, Methanamine, Benzothiazole, Methylpyrazine, 1-Butanamine, Dodecane, Dimethyldisulfide, Nonadecane, 1-Decene, 2,5-Dimethylpyrazine	Zou et al., 2007
Staphylococcus aureus	1280	Ethyleneglycol, Acetic acid, Acetone, Butanol, Ethanol, 4-Methylphenol, 2-Aminoacetophenone, 2-Pentanone, Pyrimidine, 2-Nonanone, Isopentanol	Zhu et al., 2010
Staphylococcus epidermidis	1282	Beta-Phenylethanol, Dodecanal	Kai et al., 2007
Staphylococcus spp.	1279	Acetic acid, Acetoin, Butanoic acid, Butanedione, Propanoic acid, 2-Methylpropanal, 2-Methylbutanal, 3-Methylbutanal, Pentane-2,3-dione	Schulz and Dickschat, 2007
Staphylococcus xylosus	1288	Acetic acid, Acetaldehyde, 2-Hydroxy-3-butanone, Acetone, Benzaldehyde, Butanoic acid, 3-(Methylthio)-propanoic acid, Lactic acid, 2,3-Butanedione, Ethanol, Benzeneacetaldehyde, Benzeneacetic acid, Propanoic acid, 2-Propanol, 2-Phenylethanol, 2-Methylpropanol, 2-Methylpropanal, 2-Butanone, 2-Methylpropanoic acid, 2-Methylbutanal, Acetophenone, 2-Phenylethyl acetate, 2-Methylbutanol, 3-Methylbutanal, 2,3-Pentanedione, Dimethyldisulfide, 3-(Methylthio)-propanal, 2,5-Dimethylpyrazine, 3-Methylbutanol, 3-Methyl-1-butyl acetate, 2-Methylbutanoic acid, 3-Methylbutanoic acid,, 3-Methylbut-2-en-1-ol,, 3-Methylbut-3-en-1-ol,, 2-Methyltetrahydrothiophen-3-one	Beck et al., 2002
		3-Methylmercapto propionate, Phenylacetaldehyde, Phenyl acetate, 2-Phenylethanol, Methylpropanoic acid, 2-Methylbutanoic acid, 3-Methylbutanoic acid,, 3-Methylbut-2-en-1-ol,, 3-Methylbut-3-en-1-ol,, 2-Methyltetrahydrothiophen-3-one	Schulz and Dickschat, 2007
Stappia marina	281252	Tetramethylpyrazine, 2-Ethyl-5-methylpyrazine, 5-Methyl-2-(1-methylethyl)pyrazine	Dickschat et al., 2005e
Stenotrophomonas maltophilia	40324	Acetamide, Benzaldehyde, Phenylacetaldehyde, Methanamine, Benzothiazole, Methylpyrazine, 1-Butanamine, Nonadecane, 1-Decene, 2,5-Dimethylpyrazine	Zou et al., 2007
		Propanone, Benzaldehyde, Phenol, Benzenacetaldehyde, Propionic acid, 1-Hexadecanol, Benzeneethanol, Phenylethanone, Cyclohexene, Nonane, 2-Undecanone, Decanal, Hexadecane, Tetradecane, 2-Nonanone, Terpineol, 2-Octanol, Trimethylpyrazine	Gu et al., 2007
		Dodecane, Dimethyldisulfide	Zou et al., 2007; Gu et al., 2007
Stenotrophomonas rhizophila	216778	beta-Phenylethanol	Kai et al., 2007
		Dodecanal	
Stigmatella aurantiaca	41	Benzaldehyde, Benzylalcohol, Butyl acetate, Dimethyltrisulfide, Menthol, Hexadecan-1-ol, 2-Phenylethanol, 2-Methoxy-1,1’-biphenyl, Methyl benzoate, 4-Butanolide, Acetophenone, 4-Pentanolide, Undecan-2-one, Tetradecan-1-ol, 2-Methylbutyric acid, 6-Methylhept-5-en-2-one, 3-Methylbutyric acid, para-Menth-1-en-3-ol, Butyl propionate, Pent-2-en-4-olide, Pentadecan-1-ol, Methyl 3-methyl crotonate, 2-Acetylfuran, Undecan-2-ol, beta-Copaene, Guaioxide, 13-Methyltetradecan-1-ol, Tetradecan-4-olide, N-Isopentylacetamide, N-Isopentylideneisopentylamine, 6,10-Dimethylbicyclo[4.4.0]dec-1-ene, N-Isopentylformamide, (E,E)-Farnesol, N-(2-Phenylethylidene)isopentylamine, Geranylacetone, Methyl-2-methyl crotonate, 2-Methyltetradecan-4-one, beta-Ylangene, Methyl salicylate, 4-	Dickschat et al., 2005b

Table 2 (continued)

Species	Tax ID (NCBI)	Volatile Synonym	References
		Methylquinoline, Hexahydrofarnesylacetone, Nonadecan-10-one, 5-Methylhexan-3-one, Dodecan-3-one, alpha-Terpineol, S-Methylmethanethiosulfonate, Dihydroactinidiolide, 1-Phenylundecan-1-one, 1-Phenylnonan-1-one, 1-Phenyldecan-1-one, beta-Eudesmol, 6,10-Dimethylundeca-5,9-dien-2-ol, Dodecan-3-ol, Methyl-5-methyl hexanoate, Nerolidol, Germacrene D, Valerianol, Geosmin, (1(10)E,5E)-Germacradien-11-ol	
		Methyl salicylate, 4-Methylquinoline, Hexahydrofarnesylacetone, Nonadecan-10-one, 5-Methylhexan-3-one, Dodecan-3-one, alpha-Terpineol, S-Methylmethanethiosulfonate, Dihydroactinidiolide, 1-Phenylundecan-1-one, 1-Phenylnonan-1-one, 1-Phenyldecan-1-one, beta-Eudesmol, 6,10-Dimethylundeca-5,9-dien-2-ol, Dodecan-3-ol, Methyl-5-methyl hexanoate, Nerolidol, Germacrene D, Valerianol, Menthol, 2-Methylisoborneol, S-Methylmethanethiosulfinate, N-(3-Methylbutyl)acetamide, N-3-Methylbutylidene-3-methylbutylamine, N-(3-Methylbutyl)formamide, Farnesol, N-(2-Phenylethylidene)-3-methylbutylamine, p-Meth-1-en-4-ol, Rosifoliol, Isolepidozene, Octalinhydrocarbon, Geosmin, Stigmolone, (1(10)E,5E)-Germacradien-11-ol	Schulz and Dickschat, 2007
		Geosmin	Dickschat et al., 2005c
		Stigmolone	Schulz et al., 2004; Dickschat et al., 2007
		(1(10)E,5E)-Germacradien-11-ol, Geosmin	Dickschat et al., 2005d
Stigmatella spp.	40	Methyl benzoate, 4-Butanolide, Octan-4-olide, Nonan-4-olide, Undecan-4-olide, Heptan-4-olide, 4-Pentanolide, Hexan-4-olide, Decan-4-olide, Methyl 3-methylbut-2-enoate, Dodecan-4-olide, Methyl 2-methylbut-2-enoate, Tetradecan-4-olide	Schulz and Dickschat, 2007
Streptomyces albidoflavus	1886	Acetone, 1-Butanol, 2-Phenylethanol, Isoprene, 2-Methyl-1-propanol, Cyclopentanone, 2-Methyl-1-butanol, Dimethyldisulfide, 3-Methyl-3-buten-1-ol, Dimethyltrisulfide, 3-Methyl-1-butanol, Ethanethioic acid S-methyl ester, Geosmin	Schöller et al., 2002
		Geosmin, S-Methyl thiobutyrate, S-Methyl thioacetate, S-Methyl thio-3-methylbutyrate, S-Methyl thiopropionate, Albaflavenone	Schulz and Dickschat, 2007
Streptomyces albus	1888	Acetone, 1-Butanol, Cyclopentanone, 2-Methyl-1-butanol, 2-Methylpropanoic acid methyl ester, Dimethyldisulfide, 3-Methyl-3-buten-1-ol, 2-Methylbutanoic acid methyl ester, Dimethyltrisulfide, 3-Methyl-1-butanol, Ethanethioic acid S-methyl ester, Geosmin	Schöller et al., 2002
		Camphor, 3-Methylbut-3-en-1-ol, Geosmin	Schulz and Dickschat, 2007
Streptomyces antibioticus	1890	Acetone, 1-Butanol, 2-Phenylethanol, Isoprene, 2-Methyl-1-propanol, Cyclopentanone, 2-Methyl-1-butanol, 2-Methylpropanoic acid methyl ester, 3-Methylbutanoic acid methyl ester, Methyl butyrate, Dimethyldisulfide, 3-Methyl-3-buten-1-ol, 2-Methylbutanoic acid methyl ester, Dimethyltrisulfide, 3-Methyl-1-butanol, Geosmin	Schöller et al., 2002
		Geosmin	Schulz and Dickschat, 2007
Streptomyces aureofaciens	1894	Acetone, 1-Butanol, Isoprene, 2-Methyl-1-propanol, Cyclopentanone, 2-Methyl-1-butanol, 2-Methylpropanoic acid methyl ester, 3-Methylbutanoic acid methyl ester, Methyl butyrate, Dimethyldisulfide, 2-Methylbutanoic acid methyl ester, 2-Methylisoborneol, Dimethyltrisulfide, Geosmin, 3-Methyl-1-butanol	Schöller et al., 2002
Streptomyces caviscabies	90079	Linalool, 4-Methylquinoline, 2-Aminoacetophenone, 3-Methylbut-2-en-1-ol, 3-Methylbut-3-en-1-ol, 6-Methylheptan-2-one, gamma-Muurolene, 5-Methylheptan-2-one, Dimethyltetrasulfide, S-Methyl thiobenzoate, Dimethylpentasulfide, alpha-Muurolene, (E)-4,8-Dimethylnona-1,3,7-triene, 2-Methyl-2-bornene, Isothujone, delta-Cadinene, Geraniol, Cadina-1,4-diene, cis-Calamenene, Kelsoene, alpha-Gurjunene, (1(10)E,5E)-Germacradien-11-ol, 10-Methylundecan-5-olide, 10-Methylundecan-4-olide, 10-Methyldodecan-4-olide, 10-Methyldodecan-5-olide, 10-Methylundec-2-en-4-olide, 10-Methyldodec-2-en-4-olide, 10-Methylundec-3-en-4-olide, 10-Methyldodec-3-en-4-olide, beta-Muurolene	Schulz and Dickschat, 2007
Streptomyces citreus	67288	4-Methylpent-3-en-2-one, 3-Methylpentan-2-ol, 3-Methylbutan-2-ol, 2-Acetylfuran, Limonene, Geosmin, beta-Myrcene, delta-Elemene, delta-Cadinene, Geraniol, Bicyclogermacrene, Germacrene D, beta-Gurjunene, beta-Elemene, Dihydroagarofuran, (1(10)E,5E)-Germacradien-11-ol	
Streptomyces coelicolor	1902	Acetone, 1-Butanol, Isoprene, 2-Methyl-1-propanol, Cyclopentanone, 2-Methyl-1-butanol, 2-Methylpropanoic acid methyl ester, Methyl butyrate, Dimethyldisulfide, 3-Methyl-3-buten-1-ol, 2-Methylbutanoic acid methyl ester, Dimethyltrisulfide, 3-Methyl-1-butanol, Ethanethioic acid S-methyl ester, Dimethyltetrasulfide, Geosmin	Schöller et al., 2002
		Geosmin	Dickschat et al., 2005a
		(1(10)E,5E)-Germacradien-11-ol	Schulz and Dickschat, 2007

Table 2 (continued)

Species	Tax ID (NCBI)	Volatile Synonym	References
Streptomyces diastatochromogenes	42236	Acetone, 1-Butanol, 2-Phenylethanol, Isoprene, 2-Methyl-1-propanol, Cyclopentanone, 2-Methyl-1-butanol, Dimethyldisulfide, 3-Methyl-3-buten-1-ol, 2-Methylisoborneol, Dimethyltrisulfide, Geosmin, 3-Methyl-1-butanol	Schöller et al., 2002
Streptomyces griseus	1911	Acetone, 1-Butanol, 2-Phenylethanol, Isoprene, 2-Methyl-1-propanol, Cyclopentanone, 2-Methyl-1-butanol, Dimethyldisulfide, 3-Methyl-3-buten-1-ol, 2-Methylbutanoic acid methyl ester, 2-Methylisoborneol, Dimethyltrisulfide, 3-Methyl-1-butanol, Ethanethioic acid S-methyl ester, Geosmin, Dimethyltetrasulfide	
		Alpha-Pinene, Beta-Pinene, S-Methyl thiobutyrate, Dimethyltetrasulfide	Schulz and Dickschat, 2007
		Geosmin	Dickschat et al., 2005a; Nawrath et al., 2008
		beta-Gurjunene	Dickschat et al., 2005d
Streptomyces hirsutus	35620	Acetone, 1-Butanol, 2-Phenylethanol, Isoprene, 2-Methyl-1-propanol, Cyclopentanone, 2-Methyl-1-butanol, 3-Methylbutanoic acid methyl ester, Methyl butyrate, Dimethyldisulfide, 3-Methyl-3-buten-1-ol, Dimethyltrisulfide, Geosmin, 3-Methyl-1-butanol, Ethanethioic acid S-methyl ester, Dimethyltetrasulfide	Schöller et al., 2002
Streptomyces hygroscopicus	1912	Acetone, 1-Butanol, 2-Phenylethanol, Isoprene, Cyclopentanone, 2-Methyl-1-butanol, 2-Methylpropanoic acid methyl ester, 3-Methylbutanoic acid methyl ester, Methyl butyrate, Dimethyldisulfide, 2-Methylbutanoic acid methyl ester, 2-Methylisoborneol, Dimethyltrisulfide, Geosmin, 3-Methyl-1-butanol, Dimethyltetrasulfide	
Streptomyces lateritius	67313	Propanone, Benzaldehyde, Phenol, Benzenacetaldehyde, Propionic acid, 1-Hexadecanol, Benzeneethanol, Phenylethanone, Cyclohexene, Nonane, 2-Undecanone, Decanal, Dodecane, Hexadecane, Dimethyldisulfide, Tetradecane, 2-Nonanone, Terpineol, 2-Octanol, Trimethylpyrazine	Gu et al., 2007
Streptomyces lavendulae	1914	(-)-2-Methylisoborneol	Dickschat et al., 2007
Streptomyces murinus	33900	Acetone, 1-Butanol, 2-Phenylethanol, Isoprene, 2-Methyl-1-propanol, Cyclopentanone, 2-Methyl-1-butanol, 2-Methylpropanoic acid methyl ester, Methyl butyrate, Dimethyldisulfide, 3-Methyl-3-buten-1-ol, 2-Methylbutanoic acid methyl ester, 2-Methylisoborneol, Geosmin, 3-Methyl-1-butanol	Schöller et al., 2002
Streptomyces olivaceus	47716	Acetone, 1-Butanol, 2-Phenylethanol, 2-Methyl-1-propanol, 2-Methyl-1-butanol, 2-Methylpropanoic acid methyl ester, Dimethyldisulfide, 3-Methyl-3-buten-1-ol, 2-Methylisoborneol, Dimethyltrisulfide, Geosmin, 3-Methyl-1-butanol	
Streptomyces platensis	58346	Butanoic acid 2-methyl ester, 1a,2,3,3a,4,5,6,7b-Octahydro-1,1,3a,7-tetramethyl-1H-cyclopropa[a]naphthalene, trans-1,10-Dimethyl-trans-9-decalol, 5-Methoxy-1,3-dimethyl-1H-pyrazole, 1,1,4,4-Tetramethyl-2,5-dimethylene- cyclohexane, 3,3,7,11-Tetramethyltricyclo [5.4.0.0 (4,11)] undecan-1-ol, 2-(2,4-Dimethoxybenzylidenehydrazino)-N-ethyl-2-oxo-acetamide,	Wan et al., 2008
Streptomyces rishiriensis	68264	Acetone, 1-Butanol, Isoprene, 2-Methyl-1-propanol, Cyclopentanone, 2-Methyl-1-butanol, Dimethyldisulfide, 3-Methyl-3-buten-1-ol, Dimethyltrisulfide, Geosmin, 3-Methyl-1-butanol	Schöller et al., 2002
Streptomyces spp.	1883	Acetoin, Butane-2,3-diol, Isoprene, Methyl benzoate, 2-(Hydroxymethyl)furan, Hexanoic acid, 3-Methylfuran, S-Methyl thioacetate, Pentalenene, Germacrene A, Protoilludene, Benzylalcohol, Benzothiazole, 2-Phenylethyl acetate, 1-Phenylpropan-2-one, Benzyl acetate, 4-Methylquinoline, 3-Methylbut-2-en-1-ol, 2-Phenylpropan-2-ol, Dimethyldisulfide, 3-Methylbut-3-en-1-ol, Dodecan-4-olide, Butylphenyl acetate, Dimethyltetrasulfide, 4-Methylhexan-1-ol, 4-Methylquinazoline, 2-Phenylethanol, 2-Methylisoborneol, Dimethyltrisulfide, Geosmin	Schulz and Dickschat, 2007
		Acetone, 1-Butanol, Isoprene, Cyclopentanone, 2-Methyl-1-butanol, 3-Methyl-1-butanol, 2-Phenylethanol, 2-Methylisoborneol, Dimethyltrisulfide, Geosmin	Schöller et al., 2002
		Vinylguaiacol, Hexadecanoic acid, Octadecanoic acid, Methyl-4-hydroxy benzoate, Heptadecanoic acid, Tetradecanoic acid, Pentadecanoic acid, 12-Methyltetradecanoic acid, 14-Methylhexadecanoic acid, 14-Methylpentadecanoic acid, 13-Methyltetradecanoic acid, 15-Methylhexadecanoic acid, 12-Methyltridecanoic acid, Pentadecenoic acid, 9-Octadecenoic acid, Squalene, 9-Hexadecenoic acid, (R)-10-methyl-6-undecanolide, (6R,10S)-10-methyl-6-dodecanolide, 2-Phenylethanol, Geosmin	Stritzke et al., 2004
		Benzophenone, Isobornyl acetate, Linalool, Ethyl-2-methyl propionate, Methylpyrazine, Heptan-2-one, Hexan-1-ol, Benzylcyanide, 6-Methylhept-5-en-2-one, 2-Aminoacetophenone, 6-Methylheptan-2-one, 2-Acetylfuran, gamma-Muurolene, Heptane-2,5-dione, Geranylacetone, 5-Methylheptan-2-one, Butyl acetate, Cyclooctasulfur, S-Methyl thiobenzoate, Dimethylpentasulfide, Guaioxide, Methyl methylthiomethyl disulfide, alpha-Muurolene, Isolongifolene, Citronellylacetone, (E)-4,8-Dimethylnona-1,3,7-triene, 2-Methyl-2-bornene, Isothujone, delta-Cadinene, 1-epi-	Dickschat et al., 2005d

Table 2 (continued)

Species	Tax ID (NCBI)	Volatile Synonym	References
		Cubenol, 2-Methoxy-3-(1-methylpropyl)pyrazine, Geraniol, Cadina-1,4-diene, cis-Calamenene, beta-Gurjunene, Kelsoene, alpha-Gurjunene, 10-Methylundecan-5-olide, 10-Methylundecan-4-olide, 10-Methyldodecan-4-olide, 10-Methyldodecan-5-olide, 10-Methylundec-2-en-4-olide, 10-Methyldodec-2-en-4-olide, 10-Methylundec-3-en-4-olide, 10-Methyldodec-3-en-4-olide, beta-Muurolene, Benzylalcohol, Benzothiazole, 2-Phenylethyl acetate, 1-Phenylpropan-2-one, Benzyl acetate, 4-Methylquinoline, 3-Methylbut-2-en-1-ol, 2-Phenylpropan-2-ol, Dimethyldisulfide, 3-Methylbut-3-en-1-ol, Dodecan-4-olide, Butylphenyl acetate, Dimethyltetrasulfide, 4-Methylhexan-1-ol, 4-Methylquinazoline, 2-Phenylethanol, 2-Methylisoborneol, Dimethyltrisulfide, (1(10)*E*,5*E*)-Germacradien-11-ol, Geosmin	
		Ethylene	Stotzky and Schenk, 1976
		Butyrolactone	Ryan and Dow JM, 2008
		Geosmin	Dickschat et al., 2007
		(1(10)*E*,5*E*)-Germacradien-11-ol, 8,10-Dimethyl-1-octalin	Nawrath et al., 2008
Streptomyces sulphureus	47758	Geosmin	Schulz and Dickschat, 2007
Streptomyces thermoviolaceus	1952	Acetone, 1-Butanol, 2-Phenylethanol, Isoprene, 2-Methyl-1-propanol, Cyclopentanone, 2-Methyl-1-butanol, 2-Methylpropanoic acid methyl ester, Methylbutyrate, Dimethyldisulfide, 3-Methyl-3-buten-1-ol, 2-Methylbutanoic acid methyl ester, Dimethyltrisulfide, Geosmin, 3-Methyl-1-butanol, Ethanethioic acid S-methyl ester	Schöller et al., 2002
Sulfitobacter dubius	218673	Tetramethylpyrazine, 3-Ethyl-2,5-dimethylpyrazine, 2,5-Dimethyl-3-(3-methylbutyl)pyrazine, 3-Butyl-2,5-dimethylpyrazine	Dickschat et al., 2005e
Sulfitobacter pontiacus	60137	Tetramethylpyrazine, 2-Ethyl-5-methylpyrazine, 3-Ethyl-2,5-dimethylpyrazine, Ethyltrimethylpyrazine, 5-Methyl-2-(1-methylethyl)pyrazine, 2,5-Dimethyl-3-(3-methylbutyl)pyrazine, 2,5-Dimethyl-3-(2-methylpropyl)pyrazine, 3-Butyl-2,5-dimethylpyrazine, 2,5-Dimethyl-3-methylsulfanylpyrazine	
		2,5-Dimethyl-3-methylsulfanylpyrazine	Schulz and Dickschat, 2007
Sulfitobacter spp.	60136	2-Ethyl-5-methylpyrazine, 3-Ethyl-2,5-dimethylpyrazine, 5-Methyl-2-(1-methylethyl)pyrazine	Dickschat et al., 2005e
		2-(3-Methylbutyl)-3,6-dimethylpyrazine, 2-Isobutyl-3,6-dimethylpyrazine, 2-Butyl-3,6-dimethylpyrazine, 2-(2-Methylbutyl)-3,6-dimethylpyrazine, 2,5-Dimethyl-3-methylsulfanylpyrazine	Schulz and Dickschat, 2007
Thermoactinomyces spp.	2023	Isoprene, 2-Methylfuran	
Thermomonospora fusca	2021	5-Methylhexan-3-one, S-Methyl thiobutyrate	
Thermomonospora spp.	2019	Isoprene	
Tolypothrix distorta	119534	Octanal, alpha-Pinene, 2-Heptanone, Decanal, 6-Methyl-5-hepten-2-one, beta-Cyclocitral, 2-Tridecanone, Heptadecane, 2-Decanone, 6-Methylheptan-2-one, Limonene, Heptadecene, Geosmin, 7-Methylheptadecane, Nonanal, 1-Octen-3-one, 8-Methylheptadecane, beta-Ionone, 2-Decenal, beta-Ionone-5,6-epoxide	Höckelmann et al., 2004
Tolypothrix spp.	111782	Sulcatone	Schulz and Dickschat, 2007
Treponema denticola	158	Methanethiol, L-Methionine	
Variovorax spp.	34072	Methyliodide	
Veillonella spp.	29465	Acetate, Succinate, Isobutyrate, Isovalerate	Hinton and Hume, 1995
Vibrio spp.	662	Methyliodide	Schulz and Dickschat, 2007
Wolinella curva	200	Hexadecanoic acid, Dodecanoic acid, Octadecanoic acid, Tetradecanoic acid, 3-Hydroxytetradecanoic acid, 3-Hydroxyhexadecanoic acid, Hexadecenoic acid	Brondz and Olsen, 1991
Wolinella recta	203	Hexadecanoic acid, Dodecanoic acid, Octadecanoic acid, Tetradecanoic acid, 3-Hydroxytetradecanoic acid, 3-Hydroxyhexadecanoic acid, Hexadecenoic acid	
Wolinella succinogenes	844	Hexadecanoic acid, Dodecanoic acid, Octadecanoic acid, Tetradecanoic acid, 3-Hydroxytetradecanoic acid, 3-Hydroxyhexadecanoic acid, Hexadecenoic acid	
Xanthomonas campestris pv. *campestris*	340	Butyrolactone, cis-11-Methyl-2-dodecenoic acid	Ryan and Dow JM, 2008
	316273		Weise et al., 2012

Table 2 (continued)

Species	Tax ID (NCBI)	Volatile Synonym	References
Xanthomonas campestris pv. *vesicatoria* 85-10		Hexan-2-one, 2-Methylpropyl acetate, n-Octane, 5-Methylhexan-2-one, 2-Methylbutyl acetate, 3-Methylbutyl acetate, Heptan-2-one, 2,5-Dimethylpyrazine, 2-Methylpropyl propionate, n-Nonane, 6-Methylheptan-2-one, 5-Methylheptan-2-one, 2-Methylbutyl propionate, 3-Methylbutyl propionate, Octan-2-one, 2,3,5-Trimethylpyrazine, Hexyl acetate, Benzylalcohol, 3-Methylbutyl 3-methylbutyrate, Acetophenone, 7-Methyloctan-2-one, Nonan-2-one, 2-Phenylethanol, 8-Methylnonan-2-one, 7-Methylnonan-2-one, 8-Methylnonan-2-ol, 7-Methylnonan-2-ol, Decan-2-one, 2-Phenylethyl acetate, 9-Methyldecan-2-one, Undecan-2-one, Undecan-2-ol, 3,6-Dimethyl-2-(3-methylbutyl)pyrazine, 10-Methylundecan-2-one, 9-Methylundecan-2-one, 10-Methylundecan-2-ol, 9-Methylundecan-2-ol, Dodecan-2-one, Geranylacetone, 11-Methyldodecan-2-one, Tridecan-2-one, Tridecan-2-ol, 12-Methyltridecan-2-one, 11-Methyltridecan-2-one, 12-Methyltridecan-2-ol, 11-Methyltridecan-2-ol, Tetradecan-2-ol, 13-Methyltetradecan-2-one, Pentadecen-2-one, Pentadecan-2-one, 14-Methylpentadecan-2-one, 13-Methylpentadecan-2-one	
Zoogloea spp.	349	Methyliodide	Schulz and Dickschat, 2007

The name of the volatile producing species corresponds to a taxonomy ID (http://www.ncbi.nlm.nih.gov/taxonomy)

2005a; von Reuss et al., 2010). It is, for example, not known whether these volatiles act as communication signals or are used as carbon sources. Important future tasks are, therefore, elucidation of the plethora of bacterial and fungal VOCs and determination of their chemical structures and biological and ecological roles.

Volatile Mediated Bacterial-Fungal Interactions

Bacterial and fungal volatiles may play multiple roles in microbial communities belowground. Although volatiles can serve as nutrient sources, under highly competitive but symbiotic conditions they are particularly important for antibiosis and signaling, and may serve as regulative principles in any ecosystem. Subsequently, interactions between bacteria and fungi can be beneficial or detrimental. In the latter situation, the term microbiostasis is used to describe the inability of bacteria and/or fungi to multiply in natural soils (Ho and Ko, 1982). Although nutrient depletion or suboptimal environmental conditions also may account for this effect, the involvement of microbial biogenic inhibitors, including volatiles, in microbiosis is widely accepted (Hora and Baker, 1972; Griffin et al., 1975; Stotzky and Schenck, 1976 and citations therein; Chuankun et al., 2004; Zou et al., 2007; Garbeva et al., 2011). The role of volatiles in signaling events within microbial communities has not yet been well-studied. Wheatley (2002) described volatiles as info-chemicals that could mediate bacterial and fungal interactions. This was also proposed by Bending et al. (2006) for the mycorrhizal community. Fungi and plants produce volatile signal molecules that bacteria in the mycorrhizhospere may also synthesize, thereby affecting mycorrhiza formation. A similar situation has been described for the rhizobacterial community (Chernin et al., 2011). Volatiles of *Pseudomonas fluorescens* and *Serratia plymuthica* inhibited quorum-sensing in various other bacteria such as *Agrobacterium*, *Chromobacterium*, *Pectobacterium*, and *Pseudomonas* due to suppression of the transcription of N-acyl-homoserine lactone synthase genes.

Effects of Bacterial Volatiles on Fungi

Influence of Bacterial Volatiles on Germination and Mycelial Growth

The phenomenon of fungistasis was first described by Dobbs and Hinson (1953), which can be due to the negative influence of bacterial volatiles on germination and growth of soil-borne fungi. McCain (1966) showed that volatiles produced by *Streptomyces griseus* induced early sclerotia formation in *Sclerotium cepivorum* and *Rhizoctonia solani*, and

Table 3 Compilation of VOC producing fungi

Species	Tax ID (NCBI)	VOC Synonym	References
Acremonium obclavatum	132114	Acetone, Benzene, Ethanol, 2-Butanone, 2-Ethylhexanol, Pentane, Cyclohexane, Limonene, Arsenous acid, Cyclotrisiloxane	Ezeonu et al., 1994
Agaricus bisporus	5341	Isovaleric acid	Stotzky and Schenk, 1976
Agaricus campestris	56157	2,3-Dimethyl-1-pentene	
Alternaria alternata	5599	2-Methylpropanol, 2-Ethylhexanol, 2-Methylbutanol, 1-Octen-3-ol, 3-Methylbutanol, Methyl-2-ethyl hexanoate, 3-Octanone	van Lancker et al., 2008
Alternaria spp.	5598	1-Octen-3-ol, 3-Methylbutanol, 3-Octanone, 2-Octen-1-ol	Kaminski et al., 1974
		Ethylbenzene, 1-Penten-3-ol, 2-Methyl-1-propanol, 2-Methyl-1-butanol, 3-Methylfuran, Thujopsene, Dimethylbenzene	Börjesson et al., 1992
		Methoxybenzene, 1-Ethyl-2-methylbenzene, 1-Octen-3-ol, 3-Methyl-1-heptene, 2-Methyl-1-propanol, 2-Methyl-1-butanol, 3-Methyl-1-butanol, Hexanoic acid ethyl ester, 3-Cyclohepten-1-one, 3-Octanone, 2,3,5-Trimethylfuran, 1,3,6-Octatriene	Fischer et al., 1999
Aspergillus clavatus	5057	Ethylene	Stotzky and Schenk, 1976
Aspergillus flavus	5059	1-Octanol, 3-Octanol, 1-Octen-3-ol, 3-Methylbutanol, 3-Octanone	
		Nitromethane, 2-Methyl-1-propanol, Ethylbenzene, 1-Penten-3-ol, 3-Methylfuran, Limonene, Thujopsene, 1,3-Octadiene, Dimethylbenzene	Börjesson et al., 1992
Aspergillus fumigatus	746128	2-Methyl-1-propanol, Camphene, Alpha-Pinene, 2-Methyl-1-butanol, 3-Methyl-1-butanol, Citronellol, Limonene, alpha-Farnesene, trans-beta-Farnesene	Fischer et al., 1999
		Methylbenzoate, 2-Ethyl-1-hexanol, 2-Pentanone, 2-Heptanone, Butoxyethoxyethanol, 3-Octanol, 2-Nonanone, 1-Octen-3-ol, 2-Pentanol, 3-Octanone, 2-Nonen-1-ol, 3-Methyl-1-butanol	Matysika et al., 2008
Aspergillus glaucus	40379	1-Octen-3-ol, 2,4-Pentadione, 3-Octanone	Menetrez and Foarde, 2002
Aspergillus niger	5061	2-Methyl-1-propanol, Styrene, 2-Pentanone, Ethylacetate, 1,3-Pentadiene, 2-Pentanol, 3-Methyl-1-butanol	Nieminen et al., 2008
		2-Methyl-1-propanol, 2-Pentanone, 2-Heptanone, 3-Octanol, 3-Octanone, 3-Methylfuran, 1-Octen-3-ol, Pentadecene, Ethyltiglate, 1,3-Nonadiene, iso-Amyltiglate, 2-Pentanol, 3-Methyl-1-butanol	Matysika et al., 2008
		3-Octanol, 1-Octen-3-ol, 3-Methylbutanol, 2-Octen-1-ol, 3-Octanone	Kaminski et al., 1974
Aspergillus ochraceus	40380	1-Octanol, 3-Octanol, 1-Octen-3-ol, 3-Methylbutanol, 3-Octanone, 2-Octen-1-ol	Kaminski et al., 1974
Aspergillus oryzae	5062		
Aspergillus parasiticus	5067		
Aspergillus spp.	5052	Dimethylselenide	Stotzky and Schenk, 1976
Aspergillus versicolor	46472	Benzene, Ethanol, Methylbenzene, Cyclotetrasiloxane, Xylene, Cyclotrisiloxane, 2-Ethylhexanol, 1,3-Dimethoxybenzene, Limonene	Ezeonu et al., 1994
		Ethylbenzene, 1-Penten-3-ol, Thujopsene, Dimethylbenzene, 3-Methylfuran, Limonene, 2-Methyl-1-propanol, 3-Methyl-1-butanol, 1,3-Octadiene	Börjesson et al., 1992
		Anisole, 1-Octene, 3-Methoxyanisole, 3-Methyl-2-butanone, 3-Methyl-2-pentanone, Dimethyldisulfide, 3-Methyl-3-buten-1-ol, 1,3-Pentadiene, 4-Methyl-3-hexanone, 3-Octanone, 3-Methylfuran, 2-Ethylhexanol, 3-Octanol, 1-Octen-3-ol, 2-Methyl-1-propanol, 5-Ethyl-4-methyl-3-heptanone	Sunesson et al., 1995
		2-Ethyl-1-hexanol, 2-Pentanone, 2-Heptanone, 2,6-di-tert-Butyl-p-benzoquinone, 2-Nonanone, 2-Pentanol, 3-Octanone, 1,3-Dimethoxybenzene, 3-Octanol, 1-Octen-3-ol, 1,3-Octadiene, 5-Ethyl-4-methyl-3-heptanone	Matysika et al., 2008
		2-Methyl-1-butanol, 6-Methyl-2-heptanone, alpha-Muurolene, γ- Curcumene, 1-Octen-3-ol Limonene, 3-Methyl-1-butanol	Fischer et al., 1999

Table 3 (continued)

Species	Tax ID (NCBI)	VOC Synonym	References
		2-Methylbutanol, 3-Methylbutanol, Methyl-2-ethyl hexanoate, 2-Ethylhexanol, 1-Octen-3-ol, 1,3-Octadiene	van Lancker et al., 2008
		1,3-Dimethoxybenzene, 3-Octanol, 1-Octen-3-ol	Menetrez and Foarde, 2002
		2-Methyl-1-propanol	Wilkins et al., 2000
Aureobasidium pullulans	5580	Ethanol	Stotzky and Schenk, 1976
Bjerkandera adusta	5331	Phenyl pyruvate, 2-Hydroxy-3-phenylpropionic acid, Cinnamic acid,	Schulz and Dickschat, 2007
Blastomyces dermatitidis	5039	Ethylene	Stotzky and Schenk, 1976
Boletus variegatus	48592	Acetoin, Ethanol, Isobutanol, Isobutyric acid, 3-Methylbutanol	
Candida humicola	109387	Dimethylselenide, Trimethylarsine, Dimethylarsine	
Candida tropicalis	5482	Acetic acid, Acetaldehyde, Acetone, 1-Butanol, Ethanol, Methanethiol, Methanol, 2-Butanone, 2-Methyl-1-butanol	Bunge et al., 2008
Cephalosporium spp.	81097	3-Octanol, 1-Octen-3-ol, 3-Methylbutanol, 3-Octanone, 2-Octen-1-ol	Kaminski et al., 1974
Ceratocystis fagacearum	72029	Acetaldehyde, Ethyl propionate, Propyl acetate, Isobutyl acetate, Methyl isovalerate, Methyl butyrate, Butyl acetate	Lin and Phelan, 1992
Ceratocystis fimbriata	5158	Ethylene	Stotzky and Schenk, 1976
Ceratocystis spp.	5157	Acetone, Ethanol, Formaldehyde, 2-Methylpropanal, 2-Methylbutanal, Furfural, 2-Heptanone, Ethyl acetate, 2-Hexenal	
Cladosporium cladosporoides	29917	Phenylethylalcohol, 1-Pentanol, 2-Pentanone, 2-Heptanone, 1-Octene, 3-Octanol, 3-Methyl-3-buten-1-ol, 1-Octen-3-ol, 2-Pentanol, Pentadecene, 3-Methyl-1-butanol, 3-Octanone, 1,3-Nonadiene, Tetradecene	Matysika et al., 2008
		3-Pentanone, 1-Octene, 3-Methylfuran	Sunesson et al., 1995
Cladosporium sphaerospermum	92950	Alpha-Humulene, Tetramethyltetrahydrofuran	Menetrez and Foarde, 2002
Cladosporium spp.	5498	2-Pentanone	Nieminen et al., 2008
Daedalea juniperina	239201	Anisaldehyde	Stotzky and Schenk, 1976
Dipodascus aggregatus	44075	Ethyl propionate, Ethyl acetate	
Emericella nidulans	162425	2-Methyl-1-propanol, 2-Methyl-1-butanol, alpha-Terpinolene, 1-Octen-3-ol, Limonene, 3-Methyl-1-butanol, 2,3-Dimethyl-butanoic acid methyl ester, Cyclooctene	Fischer et al., 1999
Emericella spp.	5071	Styrene, 2-Pentanone, 2-Heptanone, 2-Pentanol, 3-Methyl-1-butanol, 1,3-Pentadiene	Nieminen et al., 2008
Fistulina hepatica (Schaeffer: Fr.)	40457	Benzaldehyde, 1-Butanol, Butanoic acid, Octanal, 1-Octanol, Hexadecanoic acid, Phenylacetaldehyde, Phenylacetic acid, 1,8-Cineole, Decanoic acid, Hexanal, Linalool, 2-Methyl-1-propanol, 2-Methyl-propanoic acid, Benzoic acid methyl ester, 2-Methyl-pentanoic acid, 2-Ethyl-1-hexanol, Citronellal, Pentanoic acid, Nonanoic acid, 1-Dodecanol, Methyl stearate, Hexanoic acid, 6-Methyl-5-hepten-2-one, Sabinene, 1-Octen-3-ol, Limonene, Isopropyl dodecanoate, 4-Hydroxy-4-methyl-2-pentanone, Isoamylalcohol, 1-Octen-3-one, (+)-Cuparene, Bisabololoxide B, (E)-2-Methyl-2-butenoic acid, 3-Octanone, Cinnamicaldehyde, (Z)-2-Methyl-2-butenoic acid, (E)-2-Heptenal, (E)-2-Octenal, (E)-Nerolidol	Wu et al., 2005
Fomes annosus	13563	Hexa-1,3,5-triene	Stotzky and Schenk, 1976
Fomes pomaceus	123902	Methylbromides	
Fomes spp.	40441	Ethanol, Methylbromides, Methylchloride, Isobutanol	
Fusarium sambucinum	5128	Beta-Santalene, beta-Himachalene, beta-Chamigrene, alpha-Bergamotene, Acoradiene, Ar-Curcumene, Elixene, Trichodiene, Longifolene, beta-Bisabolene, beta-Selinene, Di-epi-alpha-Cedrene, alpha-Farnesene, beta-Farnesene	Jelen et al., 1995

Table 3 (continued)

Species	Tax ID (NCBI)	VOC Synonym	References
Fusarium spp.	5506	1-Octen-3-ol, 3-Methylbutanol, 3-Octanone	Kaminski et al., 1974
Mucor hiemalis	64493	Ethylene	Stotzky and Schenk, 1976
Mucor plumbeus	97098	2-Methyl-1-propanol, 2-Pentanone, Ethyl acetate, Ethyl-2-methyl butyrate, 3-Methyl-1-butanol	Nieminen et al., 2008
Muscodor albus	152623	Acetone, Phenylethylalcohol, 2-Methyl-1-propanol,, 2-Butanone, Acetic acid, methyl ester, 2-Methyl-propanoic acid, Tetrahydrofuran,, Acetic acid ethyl ester, 2-Methylfuran, 2-Methylpropanoic acid methyl ester, 3-Methyl-1-butanol acetate, 4-Nonanone, Aciphyllene, Caryophyllene	Atmosukarto et al., 2005
Muscodor fengyangensis	910323	Phenylethylalcohol, 2-Methyl-propanoic acid, 3-Methyl-6-(1-methylethyl)- 2-cyclohexen-1-one, alpha-Phellandrene, Acetic acid 2-phenylethyl ester, Acetic acid 2-methylpropyl ester, 1-Ethenyl-1-methyl-2,4-bis(1-methylethenyl)-,[1S-(1-alpha,2-beta.,4-beta)]- cyclohexane, 2-Methylpropanoic acid methyl ester, beta-Phellandrene, Caryophylleneoxide, 3-Methyl-1-butanol, 3-Methyl-1-butanol acetate, 3,5-Dimethoxytoluene, 2-Cyclohexen-1-ol, cis-3-Methyl-6-(1-methylethyl)-2-Cyclohexen-1-ol, trans-3-methyl-6-(1-methylethyl)-2-Cyclohexen-1-ol, 2,6-Dimethyl-6-(4-methyl-3-pentenyl)-bicyclo[3.1.1]hept-2-ene, 2,6-Dimethyl-6-(4-methyl-3-pentenyl)-bicyclo[3.1.1]hept-2-ene, 1-Methyl-4-[5-methyl-1-methylene-4-hexenyl)-[S]- cyclohexene, cis-1-Methyl-4-(1-methylethyl)-2-cyclohexen-1-ol, Isoaromadendrene epoxide, Diepicedrene-1-oxide, 2-Methylene-4,8,8-trimethyl-4-vinyl-bicyclo[5.2.0]nonane, 3,3,7,11-Tetramethyl-tricyclo[6.3.0.0(2,4)]undec-8-ene, Caryophyllene, cis-alpha-Bisabolene, Caryophyllene-[I1]	Zhang et al., 2010
Paecilomyces variotii	45996	Acetone, 2-Pentanone, Propylacetate, 2-Heptanone, 1-Hexanol, 3-Methyl-2-pentanone, 2-Hexanone, 1-(1,1-Dimethylethyl)-4-ethylbenzene, 2-Methyl-1-propanol, 3-Methyl-1-butanol, 2,5-Dimethylfuran, 3-Methylfuran, 2,3,5-Trimethylfuran	Sunesson et al., 1996
		Octane, 2-Propanol, 2-Butanone, Methylacetate, Furan, Trimethylbenzene, 2-Methylpropyl formate, 2,4-Dimethylfuran, 1-Methylpropylformate, alpha-Curcumene, 2-Methyl-1,3-pentadiene, 2-Methyl-1-propanol, 3-Methyl-1-butanol, 2-Methyl-1-butanol, 2,5-Dimethylfuran, 3-Methylfuran, Xylene	Sunesson et al., 1995
		alpha-Phellandrene, alpha-Terpinene, beta-Phellandrene, γ-Cadinene, Myrcene, Germacrene B, neo-allo-Ocimene, Megastigma-4,6(e),8(Z)-triene, +alpha-Longipinene, 2-Methyl-1-propanol, 3-Methyl-1-butanol, 2-Methyl-1-butanol, 2,3,5-Trimethylfuran	Fischer et al., 1999
Penicillium aurantiogriseum	36655	Acetic acid, Ethanol, 1-Propanol, 2-Methyl-1-propanol, 3-Methylfuran, 1-Octen-3-ol, 3-Methyl-1-butanol, 3-Octen-2-ol	Börjesson et al., 1990
Penicillium brevicompactum	5074	Acetone, 2-Propanol, 2-Butanone, 3-Pentanone, 3-Methylfuran, 2-Methyl-1-propanol, 2-Methyl-1-butanol	Börjesson et al., 1992
		Styrene, 1-Octen-3-ol, Limonene, 3-Methyl-1-butanol, 2-Methyl-1-propanol, 2-Methyl-1-butanol	Fischer et al., 1999
Penicillium chrysogenum	5076	Acetic acid, Ethanol, 2-Propanol, Isoprene, 2-Methyl-1-propanol, 2-Butanol, 2-Butanone, alpha-Pinene, 3-Pentanone, 1-Pentene, 2-Octanone, 1-Dodecene, 2-Methyl-1-butanol, 2-Heptanol, 3-Pentanol, 2-Hexanone, 1-Hexene, Dimethyldisulfide, 2-Hexanol, 2-Nonanol, 1-Undecene, Methyl-2-methylbutyrate, beta-Pinene, 1-Tridecene, 2-Octanol, Nonadiene, 2-Pentanone, 3-Methyl-1-butanol 2-Heptanone, 1-Octene, 1-Heptene, 2-Nonanone, 1-Pentadecene, 1-Nonene, 1-Octen-3-ol, 1,3-Octadiene	Wilkins et al., 2000
		1-Octanol, 2-Octen-1-ol, 3-Octanol, 1-Octen-3-ol, 3-Methyl-1-butanol, 3-Octanone	Kaminski et al., 1974
		3-Methylanisole, 1,3-Dimethoxybenzene, Hexadecane, 2-Pentanol, Geosmin, 1,4-Dimethoxy-2-methyl-benzene, Ethyltiglate, 1,3-Nonadiene, iso-Amyltiglate, 2-Pentanone, 2-Heptanone, 1-Octene, 1-Heptene, 2-Nonanone, 1-Pentadecene, 1-Nonene, 2-Methylbutanol, 3-Octanol, 1-Octen-3-ol, 3-Methyl-1-butanol, 3-Octanone, 1,3-Octadiene, Tetradecene	Matysika et al., 2008
		2-Ethylhexanol, 2-Methylbutanol, 3-Octanol, 1-Octen-3-ol, 3-Methyl-1-butanol, 1,3-Octadiene	van Lancker et al., 2008
		1-Octen-3-ol, 3-Octanone, Tetradecene	Menetrez and Foarde, 2002
Penicillium citrinum	5077	1-Octanol, 3-Octanol, 1-Octen-3-ol, 3-Methylbutanol, 3-Octanone, 2-Octen-1-ol	Kaminski et al., 1974

Table 3 (continued)

Species	Tax ID (NCBI)	VOC Synonym	References
Penicillium clavigerum	29841	2-Methyl-1-propanol, alpha-Terpinene, Styrene, 2-Methyl-1-butanol, Dimethyldisulfide, Limonene, Myrcene, 3-Methyl-1-butanol, Bicyclo-(3.2.1)-octan-2-one, beta-Caryophyllene, beta-Elemene	Fischer et al., 1999
Penicillium commune	36653	Acetone, Dimethylsulfide, Cresol, Camphene, alpha-Pinene, Ethyl propanoate, Ethyl butanoate, Propyl acetate, 2-Methylpropyl acetate, Cyclopentanone, 2-Methyl-1-butanol, Ethyl acetate, Heptane, 3-Methylfuran, beta-Pinene, Limonene, Ethyl-2-methyl butanoate, 3-Methylbutylacetate, Methyl-(1-methylethenyl)benzene, alpha-Curcumene, Ethanthioic acid-S-(2-methyl)butyl ester	Sunesson et al., 1995
		2-Ethyl-1-hexanol, 2-Pentanone, 2-Heptanone, 1-Hexanol, 2-Methyl-1-butanol, 1-Octen-3-ol, Dimethyltrisulfide, Geosmin, 1-Methoxy-4-(1-methylethyl)benzene, 1-Methoxy-3-methylbutane, 3-Octanone	Sunesson et al., 1996
		2-Methyl-1-propanol, 2-Butanone, Methyl acetate, 3-Methylanisole, Dimethyldisulfide, 2,5-Dimethylfuran, 3-Methyl-1-butanol	Sunesson et al., 1995, 1996
Penicillium crustosum	36656	2-Methyl-1-propanol, Styrene, Dodecane, 2,5-Dimethylfuran, 2-Ethylfuran, Limonene, 3-Methyl-1-butanol, 2,3,5-Trimethylfuran	Fischer et al., 1999
Penicillium cyclopium	60167	2-Methyl-1-propanol, 2,5-Dimethylfuran, γ-Cadinene, 2-Methylenebornane, 2-Methyl-2-bornene, 2,3,5-Trimethylfuran, Cyclooctene, Germacrene A	
Penicillium digitatum	36651	Ethane, Ethylene, Acetylene, Propane, Propylene	Stotzky and Schenk, 1976
Penicillium expansum	27334	1-Pentanol, 2-Methyl-1-propanol, 3-Methylanisole, 2-Ethyl-1-hexanol, 2-Pentanone, 2-Heptanone, 2-Methyl-1-butanol, 3-Octanol, Dimethyldisulfide, alpha-Terpineol, 1-Octen-3-ol, 2-Pentanol, 3-Octanone, 1,3-Octadiene	Matysika et al., 2008
		Styrene, 1-Methoxy-3-methylbenzene, Aromadendrene, Elemol, Germacrene B, γ Curcumene, Bicycloelemene	Fischer et al., 1999
		Geosmin	Mattheis and Roberts, 1992
Penicillium funiculosum	28572	1-Octen-3-ol, 3-Methylbutanol, 3-Octanone, 2-Octen-1-ol	Kaminski et al., 1974
Penicillium glabrum	69773	2-Methyl-1-propanol, 2-Butanone, 3-Pentanone, 2-Methyl-1-butanol, 3-Methylfuran, 1-Octen-3-ol, 3-Octanone, 1,3-Octadiene, Dimethylbenzene, Limonene	Börjesson et al., 1992
		Styrene, 2-Methyl-1-butanol, 3-Methyl-1-butanol, Limonene	Fischer et al., 1999
Penicillium italicum	40296	Methylbutenone, Cedrol, Cedrene	Menetrez and Foarde, 2002
Penicillium raistrickii	69783	1-Octen-3-ol, 3-Methylbutanol, 3-Octanone, 2-Octen-1-ol	Kaminski et al., 1974
Penicillium roqueforti	5082	Toluene, 2-Methylpropanoic acid, Acetic acid 2-methylpropyl ester, Heptane, 3-Methyl-1-butanol, Isoamylacetate, 1,3-Octadiene, Xylene	Jelen et al., 1995
		2-Methyl-1-propanol, 2-Methyl-1-butanol, 3-Methylfuran, Dimethylbenzene, Limonene, 3-Octanone, 1,3-Octadiene	Börjesson et al., 1992
		Alpha-Phellandrene, Styrene, 3-Octanol, Undecane, beta-Himachalene, 1-Octene-3-ol, 3-Carene, beta-Myrcene, (+)-2-carene, beta-Patchoulene, Aristolochene, Di-epi-alpha-cedrene, beta-Elemene, beta-Bisabolene, Limonene, 3-Octanone	Jelen 2003
Penicillium spp.	5073	2-Methyl-1-propanol, Styrene, 2-Pentanone, Ethylacetate, 2-Pentanol, Ethyl-2-methylbutyrate, 3-Methyl-1-butanol, 1,3-Pentadiene, 2-Heptanone	Nieminen et al., 2008
		4-Allylanisole, 2-Heptanone	Bjurman et al., 1997
		Dimethylselenide	Stotzky and Schenk, 1976
Penicillium variabile	28576	2-Ethylhexanol, 2-Heptanone, 2-Ethylhexanoic acid, 2-Methylbutanol, Terpinolene, 3-Octanol, 1-Octen-3-ol, 3-Methylbutanol, Methyl-2-ethyl hexanoate, 3-Octanone, 1,3-Octadiene	van Lancker et al., 2008
Penicillium viridicatum	60134	1-Octanol, 3-Octanol, 3-Octanone, 2-Octen-1-ol, 1-Octen-3-ol, 3-Methylbutanol	Kaminski et al., 1974

Table 3 (continued)

Species	Tax ID (NCBI)	VOC Synonym	References
		2-Ethylhexanol, 2-Methylbutanol, 2-Ethylfuran, 2-Pentylfuran, 1,3-Octadiene, 1-Octen-3-ol, 3-Methylbutanol	van Lancker et al., 2008
Phellinus spp.	40470	Benzylalcohol, Methyl salicylate, Phenylethylalcohol, Methyl benzoate, Ethyl benzoate	Stotzky and Schenk, 1976
Phialophora fastigiata	91935	Acetone, 2-Butanone, Methyl benzoate, Cyclopentanone, Methyl-3-methyl butanoate, Dimethyldisulfide, 1-Octen-3-ol, 3-Methyl-1-butanol, 3-Octanone, Caryophyllene	Sunesson et al., 1995
Puccinia graminis var. tritici	5297	Acetaldehyde, Furfural, Trimethylethylene, n-Nonanal, Methyl ferulate	Stotzky and Schenk, 1976
Rhodotorula glutinis	5535	Phenylethylalcohol, 2-Methyl-1-propanol, 3-Methyl-2-butanol	Menetrez and Foarde, 2002
Saccharomyces cerevisiae	4932	Acetic acid, 2-Propanone, 1,2-Benzenedicarboxylic acid, 2-Propanol, Phenylethylalcohol, 2-Methyl-1-propanol, 2-Butanone, 2-Methyl-propanoic acid, 1,2-Dimethylbenzene, 2-Ethyl-1-hexanol, 2-Pentanone, Acetic acid ethenyl ester, 2-Methyl-butanoic acid, Pyrazine, 3-Methyl-butanoic acid, Dimethyldisulfide, Undecane, Limonene, 2,5-Dimethylpyrazine, Ethanol, Ethylacetate	Bruce et al., 2004
		Acetaldehyde, n-Propanol, Isobutanol, Ethanol, Ethylacetate	Stotzky and Schenk, 1976
Schizophyllum commune	5334	Methylmercaptan, Dimethylselenide	Stotzky and Schenk, 1976
Scopulariopsis brevicaulis	40375	Dimethylselenide, Trimethylarsine, Dimethylarsine	
Stachybotrys chartarum	74722	1-Butanol, 2-Propanol, Isoprene, 2-Methyl-1-propanol, 2-Butanol, Methyl benzoate, Anisole, m-Methylanisole, Methyl-2-methyl propionate, Dimethyldisulfide, 3-Methylfuran, o-Methylanisole, Dimethylhexadiene	Wilkins et al., 2000
Staphylococcus xylosus	1288	Pyridine	Matysika et al., 2008
Thielaviopsis basicola	124036	Acetone, Ethanol, Formaldehyde, 2-Methylpropanal, 2-Methylbutanal, Furfural, 2-Heptanone, Ethyl acetate, 2-Hexenal	Stotzky and Schenk, 1976
Tilletia caries	13290	Trimethylamine	Stotzky and Schenk, 1976
Tilletia controversa	13291	Trimethylamine	
Tilletia foetida	157183	Trimethylamine	
Trichoderma atroviride	63577	Phenylethylalcohol, α-Phellandrene, γ-Terpinene, α-Terpinene, 2-Heptanone, 2-Undecanone, β-Phellandrene, α-Terpinolene, 3-Octanol, 2-Nonanone, 1-Octen-3-ol, 2-Pentylfuran, 2-n-Heptylfuran, 6-Pentyl-α-pyrone, α-Bergamotene, α-Zingiberene, 3-Octanone, β-Bisabolene, α-Curcumene, p-Menth-2-en-7-ol, α-Farnesene, β-Farnesene, Nerolidol, γ-Curcumene, β-Sesquiphellandrene	Stoppacher et al., 2010
Trichoderma aureoviride	64502	Acetaldehyde, Acetone, Benzylalcohol, Chloroform, Isobutane, 2-Methyl-1-propanol, 2-Ethyl-4-methyl-1-pentanol, Heptanone, Nonane, Heptane, Decane, 2,4-Dimethylheptane, 3-Methyl-2-hexanol, 7-Octen-4-ol, 5-Methyl-5-hexen-3-ol, 1,3-Hexadien-5-yne	Bruce et al., 2000
Trichoderma pseudokoningii	317029	2-Propanone, Butanal, 1-Butanol, Octane, 1-Propanol, Hexanal, 1-Pentanol, Acetonitrile, 2-Methyl-1-propanol, 2-Butanone, p-Xylene, 2-Methylpentane, Methyl-cyclohexane, Hexane, n-hexane, 2-Octanone, 1-Hexanol, Heptanal, Formic acid heptyl ester, Decanal, 2-Methyl-butanol, Acetic acid ethyl ester, Heptane, Limonene, 3-Methyl-1-butanol, 2-Propyl-1-pentanol, 2-Octen-1-ol, 2,2,4,6,6,-Pentamethyl-3-heptene, 2-Propenylidene-cyclobutene	Wheatley et al., 1997
Trichoderma spp.	5543	Acetaldehyde, Acetone, Ethanol	Stotzky and Schenck, 1976
		Styrene, 2-Pentanone, 3-Methyl-1-butanol, 1,3-Pentadiene	Nieminen et al., 2008
Trichoderma viride	5547	2-Propanone, Benzaldehyde, Butanal, 1-Butanol, Octane, Propanal, 1-Propanol, Isopropyl-alcohol, Hexanal, Acetonitrile, 2-Butanone, Benzothiazole, p-Xylene, Methyl-cyclohexane, 2-Heptanone, Hexane, 2-Octanone, Heptanal, Decanal, 2-Methyl-1-butanol, Acetic acid 2-ethyl ester, Heptane, 6-Methyl-5-hepten-2-one, 3-Methylhexane, 4-Penten-2-ol, Pentadecane, 3-Methyl-1-butanol, Nonanal, 2-Propyl-1-pentanol, 2,4,6-Trimethyl-1-nonene, Caryophyllene, 2-Methyl-1-propanol	Wheatley et al., 1997

Table 3 (continued)

Species	Tax ID (NCBI)	VOC Synonym	References
		2-Propanol, 1-Pentanol, 2-Hexanone, 3-Methylfuran, 2-Methyl-1-propanol	Wilkins et al., 2000
Tuber aestivum	59557	Acetaldehyde, Acetone (2-Propanone), 1-Butanol, Ethanol, 1-Propanol, Dimethylsulfide, 2-Methyl-1-propanol, 2-Butanol, 2-Butanone, Acetic acid methyl ester, Propanoic acid ethyl ester, Propanoic acid propyl ester, 2-Methyl-1-butanol, Acetic acid ethyl ester, Butanoic acid methyl ester, Acetic acid 2-methylbutyl ester, 4-Hydroxy-3-methyl-2-butanone, 2-Methyl-ethyl-butanoic acid	March et al., 2006
Tuber brumale	60458	Acetaldehyde, Acetone (2-Propanone), 1-Butanol, Ethanol, Isopropylalcohol, 2-Methyl-1-propanol, 2-Butanol, 2-Butanone, Acetic acid methyl ester, 1-Methoxy-3-methylbenzene, Propanoic acid ethyl ester, Butanoic acid ethyl ester, Butanoic acid propyl ester, Propanoic acid propyl ester, 2-Methyl-1-butanol, Acetic acid ethyl ester, Butanoic acid methyl ester, Butanoic acid-1-methylpropyl ester, 4-Hydroxy-3-methyl-2-butanone, Butanoic acid 2-methyl-ethyl ester	March et al., 2006
Tuber melanosporum	39416	Acetaldehyde, Acetone (2-Propanone), 1-Butanol, 1-Propanol, Isopropylalcohol, 2-Methyl-1-propanol, Acetic acid methyl ester, Propanoic acid ethyl ester, Butanoic acid propyl ester, Propanoic acid propyl ester, Acetic acid-1-methylethyl ester, 2-Methyl-1-butanol, Acetic acid ethyl ester, Butanoic acid methyl ester, Butanoic acid 2-methyl- methyl ester, 4-Hydroxy-3-methyl-2-butanone, Butanoic acid 2-methyl-ethyl ester, Pentanoic acid 4-methyl- ethyl ester, 2-Methyl-3-ethyl-2-pentene, Ethanol, Dimethylsulfide, 2-Butanol	March et al., 2006
		2-Butanone, 3-Octanol, bis(Methylthio)methane, 1-Octen-3-ol, 1-Octen-3-one, 3-Octanone, Ethanol, Dimethylsulfide, 2-Butanol	Pelusio et al., 1995
Tuber mesentericum	92904	Acetaldehyde, Acetone (2-Propanone), 1-Butanol, Ethanol, 1-Propanol, Dimethylsulfide, 2-Methyl-1-propanol, 2-Butanol, 2-Butanone, Acetic acid methyl ester, 1-methoxy-3-methylbenzene, Propanoic acid ethyl ester, Butanoic acid propyl ester, Propanoic acid propyl ester, 2-Methylbutan-1-ol, Acetic acid ethyl ester, 3-Methyl-butanal, Butanoic acid methyl ester, 2-Methylbutyric acid methyl ester, 2-Methylbutyric acid ethyl ester, 2-Methyl-3-ethyl-2-pentene	March et al., 2006
Tuber rufum	119233	Acetaldehyde, Acetone (2-Propanone), 1-Butanol, Ethanol, Dimethylsulfide, Isopropylalcohol, 2-Butanol, 2-Butanone, Acetic acid methyl ester, Propanoic acid ethyl ester, Butanoic acid ethyl ester, Butanoic acid propyl ester, Propanoic acid propyl ester, 2-Propyl acetate, Acetic acid propyl ester, 2-Methyl-1-butanol, Acetic acid ethyl ester, Propanoic acid methyl ester, Butanoic acid methyl ester, 2-Methylbutyric acid methyl ester, 4-Hydroxy-3-methylbutan-2-one, 2-Methylbutyric acid ethyl ester, Acetic acid butyl ester, N-methylene-ethenamine	March et al., 2006
Ulocladium chartarum	119957	2-Methylpropanol, 2-Pentanone, 2-Heptanone, 2-Ethylhexanoic acid, 2-Methylbutanol, 2-Hexanone, Dimethyldisulfide, 2-Nonanone, 6-Methyl-2-heptanone, 5-Methyl-2-heptanone, 3-Methylbutanol, Methyl 2-ethyl hexanoate	van Lancker et al., 2008

The name of the volatile producing species corresponds to a taxonomy ID (http://www.ncbi.nlm.nih.gov/taxonomy) reduced sporulation in *Gloeosporium aridum*. A strong inhibition of spore germination of *Cladosporium cladosporioides* was caused by but-3-en-2-one produced by *Streptomyces griseoruber* (Herrington et al., 1987). Zou et al. (2007) screened 1080 bacterial isolates for fungistatic activity. A total of 328 isolates belonging to the family of *Rhizobiaceae*, *Xanthomonadaceae*, *Micrococcaceae*, *Alcaligenaceae*, and to the order of *Bacillales* were identified as decreasing germination and mycelial growth of *Paecilomyces lilacinus* and *Pochonia chlamydosporia*. The spore germination of both fungi also was strongly inhibited by soil direct fungistasis and soil volatile fungistasis. Both effects correlated closely with impaired spore germination and disappeared after autoclaving. Several volatiles were identified, and trimethylamine, benzaldehyde, and N,N-dimethyloctylamine showed strong antifungal activity (Chuankun et al., 2004).

In order to identify bacterial isolates specifically antagonistic to plant pathogens, many *in vitro* experiments have been done. The experimental setup had to ensure that only volatile metabolites would influence fungal growth. Split Petri dishes (Fernando et al., 2005; Kai et al., 2007; Vespermann et al., 2007), separated agar patches (Alharbi et al., 2011), or the inversion of one bottom plate over a second one (Bruce et al., 2000) assured the exchange of volatiles solely in the headspace. Vespermann et al. (2007) and Kai et al. (2007 and 2008) conducted a comprehensive investigation using *Bacillus subtilis*, *Pseudomonas fluorescens*, *Pseudomonas trivialis*, *Burkholderia cepacia*, *Staphylococcus epidermidis*, *Stenotrophomonas maltophilia*, *Stenotrophomonas rhizophila*, *Serratia odorifera*, and *Serratia plymuthica* against pathogenic fungi, including *Aspergillus niger*, *Fusarium culmorum*, *Fusarium solani*, *Microdochium bolleyi*, *Paecilomyces carneus*, *Penicillium waksmanii*, *Phoma betae*, *Phoma eupyrena*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Trichoderma strictipile*, and *Verticillium dahliae*. All rhizobacteria inhibited the mycelial growth of most fungi. The extent of inhibition depended on the individual bacteria-fungus combination. Noticeably, *Fusarium solani* turned out to be resistant against the bacterial volatiles. The spectra of bacterial volatiles produced included many unknown components; however, 2-phenylethanol, 1-undecene, dodecanal, dimethyl disulfide (DMDS), and dimethyl trisulfide (DMTS) could be identified (Kai et al., 2007). DMDS and 1-undecene indeed inhibited the growth of *F. culmorum* when applied as individual compounds in dual-culture tests (Kai et al., 2009). Several other reports also confirmed the antifungal action of volatiles produced by antagonistic rhizobacteria. *Pseudomonas fluorescens* and *Pseudomonas pumila* inhibited most effectively the growth of *Gaeumannomyces graminis* var *tritici*, the cause of take-all disease in wheat (Babaeipoor et al., 2011). *Gluconacetobacter diazotrophicus* decreased the growth of *Fusarium oxysporum* (Logeshwarn et al., 2011), *Bacillus pumilus*, *Bacillus subtilis*, and *Bacillus cereus* hindered growth of *Botrytis mali* (Jamalizadeh et al., 2010), and volatiles produced by *Bacillus subtilis* showed antifungal activity towards *Rhizoctonia solani* and *Pythium ultimum* (Fiddaman and Rossall, 1993) and *Aspergillus alternate*, *Cladosporium oxysporum*, *Fusarium oxysporum*, *Paecilomyces lilacinus*, *Paecilomyces variotii*, and *Pythium afertile* (Chaurasia et al., 2005). *Bacillus* spp. impaired the growth of *Phytophthora sojae*, which causes the soybean damping-off disease (Tehrani et al., 2002). Interestingly, the dual application of *Bacillus pumilus* and the mycorrhizal fungus *Glomus mosseae* improved the growth of mandarin plants, directly attributed in part to growth inhibition of fungal pathogens by rhizobacterial volatiles (Chakraborty et al., 2011). The volatiles 1-octen-3-ol, benzothiazol, and citronellol produced by *Paenibacillus polymyxa* strongly inhibited mycelial growth and impaired germination of eight fungal pathogens, including *Botrytis cinerea* (Zhao et al., 2011). Wan et al. (2008) investigated the effect of headspace volatiles of *Streptomyces plantesis* on phytopathogenic fungi. Two antifungal components were identified: 2-phenylethanol and a phellandrene derivative were responsible for the suppression of mycelial growth of *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *Botrytis cinerea*. Ascospore germination was suppressed up to 90 % by volatiles released by *Pseudomonas* sp., which were isolated from canola and soybean plants (Fernando et al., 2005). *Staphylococcus pasteuri* showed a significant antifungal activity *in vitro* against *Tuber borchii* and inhibited ectomycorrhizal formation (Barbieri et al., 2005).

Many *Pseudomonas* species are known to produce HCN as an effective antifungal component (Voisard et al., 1989; Haas and Défago, 2005). Although HCN production could be correlated to fungistasis, its antifungal effect often could only be verified *in vitro*. Rhizobacterial isolates were screened for HCN production and diffusible antifungal metabolites, and tested against *Verticilium dahliae* and *Rhizoctonia solani* in dual-culture tests (Tehrani et al., 2001; Afsharmanesh et al., 2006), and subsequently used in greenhouse experiments. Interestingly, HCN producers showed the highest efficiency when applied to the soil, whereas non-producers were more efficient when applied to seeds. Antifungal properties also have been attributed to gaseous ammonia. Schippers et al. (1982) showed that ammonia release from soil as well as from an ammonium sulfate solution inhibited conidia germination of *Botrytis cinerea* and *Penicillium nigricans*. However, some fungi such as *Fusarium culmorum* and *Verticillium nigrescens* were not affected by ammonia. Furthermore, other volatiles released from the soil decreased conidia germination and tube growth of these two fungi. Similarly, Howell et al. (1988) identified ammonia to be the antifungal component in dual-culture tests using *Enterobacter cloacae*, *Rhizoctonia solani*, and *Pythium ultimum*.

Fungal growth promotion by bacterial volatiles has hardly ever been reported. Mackie and Wheatley (1999) and Wheatley (2002) selected four fungi as representative of a range of several habitats and challenged them *in vitro* with headspace volatiles of a variety of randomly selected soil bacteria. The response was unique for each fungal-bacterial combination, and revealed positive, negative, as well as neutral effects on radial growth of *Trichoderma viride*, *Phanaerochaete magnoliae*, *Phytophthora cryptogea*, and *Gaeumannomyces graminis* var *tritici*. Only *P. cryptogea* exhibited a significant increase in growth upon exposure to volatiles of certain bacterial isolates.

Impact of Bacterial Volatiles on Fungal Morphology

Several reports also have focused on morphological changes in fungi following bacterial volatile treatment. Fiddaman and Rosall (1993) observed abnormal hyphae with deformation and enhanced vacuolation in *Rhizoctonia solani* and *Pythium ultimum* exposed to volatiles produced by *Bacillus subtilis*. The same bacterial species caused hyphal and conidial deformations in *Aspergillus alternaria*, *Cladosporium oxysporum*, *Fusarium osysporum*, *Paecilomyces lilacinus*, *Paecilomyces variotii*, and *Pythium afertile*. Transverse and longitudinal septae completely disappeared in *Aspergillus alternaria*, and conidia became thick-walled and irregular in shape. Conidia formation was sometimes arrested, and in *Cladosporium oxysporum*, conidiophores became vegetative and stunted. Swelling of hyphae, vacuolization, and granulation lead finally to lysis of fungal mycelium in *Fusarium oxysporum*, *Paecilomyces lilacinus*, and *Paecilomyces variotii* (Chaurasia et al., 2005). Swollen terminal cells and bulging intercalary cells also were described for *Tuber borchii* upon exposure to volatiles emitted by *Staphylococcus pasteuri* and, finally, fungal mycelium showed swollen and contorted patterns when treated with 1-octen-3-ol (Barbieri et al., 2005). Benzothiazol caused a more frequent branching of the mycelium and increased conidia production, whereas citronellol only induced a slight hyphal contortion. All three compounds were components of the volatile mix produced by *Paenibacillus polymyxa* (Zhao et al., 2011).

Influence of Bacterial Volatiles on Mycorrhizal Fungi

Mycorrhiza is a complex symbiotic community including plant roots, mycorrhizal fungi, and associated bacteria (see Jung et al., 2012, this issue). Not only their physical contact but also the release of bioactive molecules, including volatiles, apparently play a regulatory role in a mycorrhizal network establishment (Bonfante and Anca, 2009). Associated bacteria comprise primarily the mycorrhiza helper bacteria (MHB) as well as rhizobacteria with beneficial or deleterious functions (Bonfante and Anca, 2009; Miransari, 2011). In 1991, Tylka et al. demonstrated that the MHB *Streptomyces orientalis* stimulated spore germination in *Gigaspora margarita* and *Glomus mossae*. Garbaye and Duponnois (1992) proposed that MHB directly stimulate the growth of *Laccaria laccata* by releasing volatile substances. Volatiles emitted by a bacterial isolate originally associated with *Gigaspora margarita* also promoted *in vitro* host fungus growth (Horii and Ishii, 2006), and volatile and diffusible compounds produced by MHB strains obtained from *Glomus clarum* spores stimulated or arrested spore germination, dependent on the bacterial species. Complete inhibition of spore germination, however, was only related to the volatiles (Xavier and Germida, 2003). Aspray et al. (2006) revealed that stimulation of mycorrhiza formation of *Lactarius rufus* required close proximity or contact. Volatiles of the MHB *Paenibacillus* sp. alone had significant negative effects on mycorrhiza formation. Furthermore, volatiles of the MHB *Streptomyces* spp., which actually promoted growth of the ectomycorrhizal fungus *Amanita muscaria*, did not affect mycelial extension rates (Schrey et al., 2005). The antagonist *Bacillus subtilis* JA inhibited significantly the spore germination and hyphal growth of a monoxenic strain of *Glomus etunicatum* in dual-culture experiments (Xiao et al., 2008), whereas volatiles produced by *Klebsiella pneumonia* promoted hyphae extension distantly located from the germinated spores of *Glomus deserticola*. Both organisms were indigenous to the roots of sea oats (Will and Sylvia, 1990).

Impact of Bacterial Volatiles on Fungal Enzyme Activities and Gene Expression

Mackie and Wheatley (1999) and Wheatley (2002) investigated the effect of bacterial volatiles on physiological properties of fungi by monitoring laccase and tyrosinase activity of *Phanaerochaete magnoliae* and *Trichoderma viride* upon exposure to volatiles of three selected soil bacteria isolates (A, B, C). Laccase activity completely ceased in *P. magnolia* in the presence of isolates A, B, C, whereas tyrosinase activity was inhibited only by the presence of isolate B. Isolate B was the only one to affect laccase activity in *T. viride*. The observed decrease in fungal growth correlated with decreased enzyme synthesis rather than inhibition of enzyme activity (Wheatley, 2002). Laccase activity in *Rhizoctonia solani* was induced after co-cultivation with *Pseudomonas fluorescens*. Due to the experimental setup, it was not possible to distinguish between effects of diffusible and volatile metabolites (Crowe and Olsson, 2001). Inhibition of enzyme activities may also be involved in the complete loss of pigmentation after treatment of *Fusarium oxysporum* with citronellol, a compound emitted by *Paenibacillus polymyxa*

(Zhao et al., 2011). In contrast, Kai et al. (2009) observed a dark discoloration of the agar when fungi were exposed to rhizobacterial volatiles.

At present there are few reports that bacterial volatile components may affect gene expression. Minerdi et al. (2008, 2009) demonstrated an indirect volatile mediated effect of bacteria on fungal gene expression. The antagonistic wild type (WT) strain *Fusarium oxysporum* MSA35 lives in symbiosis with associated bacteria of the genera *Serratia*, *Achromobacter*, *Bacillus*, and *Stenotrophomonas*. Volatiles produced by the WT repressed the expression of two putative virulence genes of a pathogenic *Fusarium oxysporum lactucae* strain. When cured of the bacterial symbionts, the WT turned pathogenic and the sesquiterpene caryophyllene was no longer in the headspace of the cured WT. It also was not found in the headspace of the ectosymbionts, so this volatile seems to mediate a mechanism for the antagonistic properties of the *Fusarium oxysporum* WT. However, caryophyllene is only produced by the WT in the presence of the bacterial symbionts.

Possible Mechanisms of Actions of Volatiles

Presently, little is known about mechanisms of action and detoxification of bacterial volatiles in fungi. It is known that the cyanide ions from HCN are potent inhibitors of many metal-containing enzymes, in particular of copper-containing cytochrome c oxidases (Haas and Défago, 2005). However, it remains unclear how most volatiles develop their activity. One scenario relates to the production of melanin (Kai et al., 2009; Zhao et al., 2011). Melanins are known to reinforce the cell wall or accumulate on the cell surface where they develop antioxidative properties and scavenge free radicals. In fungi, melanins are synthesized via the polyketide synthase pathway (Jacobson, 2000), but phenol oxidizing enzymes such as laccases and tyrosinases may also be involved (Williamson, 1997). Intracellular laccases account for detoxification of chemicals (Champagne and Ramsay, 2010). In this regard, the increase of laccase activities reported by Crowe and Olsson (2001) might result from the presence of eligible volatile substrates, whereas the decrease in laccase and tyrosinase activity reported by Mackie and Wheatley (1999) might be a sign of impaired cell homeostasis. This again demonstrates that a deleterious bacterial volatile can be considered a toxin. Fungal cells respond to it as to any other biotic or abiotic stress factors. Whole-genome expression studies conducted in fungal model organisms including *Saccharomyces cerevisiae*, *Candida albicans*, and *Schizosaccharomyces pombe* have revealed that each species responded to environmental stress with an individual change in gene expression. Some species also expressed a common set of genes, referred to as environmental stress response (ESR) (Gasch, 2007). This can include the response to cell wall stress and/or oxidative and osmotic stress. Compounds like gaseous ammonia could be considered a stress factor, impairing cell homeostasis and triggering ESR. On the other hand, sub-inhibitory concentrations of ammonia might play a part in signaling. Ammonia released from bacterial strains has been shown to stimulate *Bacillus licheniformis* to form biofilms and pigmentation (Nijland and Burgess, 2010) and to increase the antibiotic resistance of various gram-positive and gram-negative bacteria (Bernier et al., 2011). Therefore, the ecological role of microbial volatiles may be intrinsically to serve as a signal molecule within and between species. They may also function as chemical 'manipulators' to alter central metabolic pathways, contribute to nutrient scavenging, and participate in developmental processes (Hibbing et al., 2010). Interestingly, ammonia also has been identified as a long-distance signal in *Candida albicans*, warning the colony of approaching starvation (Palková and Váhová, 2003). In this sense, the mode of actions of microbial volatiles should be assessed in more detail.

Effects of Fungal Volatiles on Bacteria

Bacteriostasis, similar to fungistasis, is the inability of bacteria to multiply in soil (Ho and Ko, 1982). Bacteriostasis is influenced by environmental factors such as nutrient supply and habitat conditions, but active volatile inhibitors also may be involved (Davis, 1976). It is known to date that bacteria produce volatiles that inhibit bacterial growth (Brown, 1973; Ko and Chow, 1977; Acea et al., 1988), and that volatiles produced by fungi also affect fungi (Stotzky and Schenck, 1976; Calvet et al., 1992; McAllister et al., 1996; Bruce et al., 2000; Martinez et al., 2004), but fungal volatiles acting on bacteria has not been reported (to the best of our knowledge).

Ecological Significance of Volatile Mediated Bacterial-Fungal Interactions

Suitable microenvironments in soils attract macro- and microbiota that colonize and form microhabitats, thereby creating dynamic microecosystems. Consequently, at least in densely and diversely populated habitats, bacteria and fungi are involved in a 'networking' community characterized by mutualism, commensalism, cooperation, antagonism, competition, and coexistence (Pal and McSpadden Gardener, 2006). Interactions between organisms can be specific or non-specific, but they are mostly multitrophic, thus keeping the microecosystem in balance. This is especially true for the mycorrhizosphere, where rhizobacteria, including plant growth promoting rhizobacteria, mingle with mycorrhizal fungi and their associated bacteria, free living bacteria and fungi, protozoa (amoeba) or metazoa (nematodes), including many phytopathogenic organisms. In this arena, interactions between bacteria and fungi could have a positive or a negative impact on third parties, which is useful if the weakened party is a pathogen and the strengthened party is a valuable member of the community. It is likely that volatile compounds are involved in these phenomena, since many bacterial volatiles affect phytopathogenic fungi directly or indirectly, i.e., as a result of bacterial-fungal interactions, pathogens are affected. In any case, the plant would benefit. An elucidation of this plant-fungus-bacterium network of interactions opens the way for biological control of plant diseases. An impressive example was given by Cao et al. (2011). They showed *in vitro* and *in vivo* that a GFP-tagged *Bacillus subtilis* strain, originally isolated from the rhizosphere of a non-infested cucumber plant, was able to successfully suppress the growth of *Fusarium oxysporum* f. sp. *cucumerinum* by colonizing the root and persisting on the rhizoplane, which is critical for an effective biocontrol in this case of cucumber wilt. Although not explicitly investigated, the authors proposed antibiosis caused by diffusible agents to be at least one mode of action. This, however, does not exclude volatile agents. Other experiments with a *B. subtilis* strain isolated from the rhizosphere of wheat and soybean showed that bacterial volatiles were involved in the biocontrol of *Botrytis mali* and *Phytophthera sojae*, respectively (Tehrani et al., 2002; Jamalizadeh et al., 2010). However, when using rhizobacteria as biocontrol agents, it is apparently important that the biocontrol strain is indigenous to the treated plant species in order to prevent damage of indigenous beneficial fungi (Will and Sylvia, 1990; Xiao et al., 2008).

Volatiles also might be involved in tritrophic interactions comprising bacteria, fungi, and nematodes. *Paenibacillus polymyxa* and *P. lentimorbus* exhibited strong antifungal activities, thereby interfering with the nematode-fungus interaction *Meloidogyne incognita* - *Fusarium oxysporum*, which significantly reduced nematode infestation of tomato plants (Son et al., 2009). In addition, soil bacteria, including one rhizobacterial strain, enhanced the nematophagous activity of the nematode-trapping fungus *Arthrobotrys oligospora* by increasing trap formation and predaceous activity (Duponnois et al., 1998). Volatile signaling cannot be excluded for either experiment.

In their entirety, the emission patterns of volatile metabolites of a belowground microecosystem reflect the dynamics of the community (McNeal and Herbert, 2009). Variations could be related to changes in the microenvironment such as pH, humidity, temperature, nutrient supply, and resulting changes in metabolic activities of micro- and macrobiota. In this respect, *in vitro* studies of volatile-mediated interactions between bacteria and fungi provide only limited access to the overall picture. Artificial test conditions might produce results that cannot be postulated uncritically for natural conditions. This especially applies to artificial growth media and nutrient supplies that influence metabolic activities as well as to “out of range” concentrations of the volatile mediators emitted (Nannipieri et al., 2003; Blom et al., 2011a). The crucial question is: are these concentrations found in the habitat? Since measurements of volatile concentrations in microhabitats are presently not available, *in vitro* testing is a useful tool to reveal substantial relationships between certain partners that might come into contact in a microecosystem. The consideration of environmental conditions and the verification of *in vitro* derived results in *in situ/in natura* experiments will give an overall picture regarding the role of volatiles in bacterial-fungal interactions and the implications of these interactions in community networks.

Conclusion and Perspectives

Volatiles are only a small proportion of the total number of metabolites produced by living organisms. However, because of their unique properties they are predestined to act as infochemicals in intra- and interspecies communications in the atmosphere as well as in the soil. This paper describes the wealth of microbial volatile emissions. The number of microbial volatiles (presently comprising around 800 compounds) and presumably of those with novel structures will increase significantly as this new research field expands. Just consider i) the large number of bacteria and fungi whose volatile profiles have yet not been obtained, ii) the various growth conditions that determine and alter the VOC profiles, and iii) the huge number of not yet identified or isolated microbes (10^6!!). This foreshadows the potential this research area has and where it may develop in the future. It seems very likely that only the “tip of the iceberg” of possible ‘volatile-wired’ interactions between underground bacteria and fungi (and elsewhere) has been seen. It will be a central task in the future to elucidate the plethora of bacterial and fungal VOCs and determine their biological and ecological roles in the soil. It also is quite likely that the naturally produced VOCs can be used as potent non-invasive indicators to study soil microbial ecosystems, including far-reaching spatiotemporal dynamics and environmental perturbations. Ultimately, these microbial volatiles – individually or in mixtures, chemically synthesized or biologically emitted - with their positive and/or negative effects on other organisms may develop into useful agricultural tools.

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REVIEW ARTICLE

Quorum Sensing of Bacteria and Trans-Kingdom Interactions of *N*-Acyl Homoserine Lactones with Eukaryotes

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Abstract Many environmental and interactive important traits of bacteria, such as antibiotic, siderophore or exoenzyme (like cellulose, pectinase) production, virulence factors of pathogens, as well as symbiotic interactions, are regulated in a population density-dependent manner by using small signaling molecules. This phenomenon, called quorum sensing (QS), is widespread among bacteria. Many different bacterial species are communicating or "speaking" through diffusible small molecules. The production often is sophisticatedly regulated via an autoinducing mechanism. A good example is the production of N-acyl homoserine lactones (AHL), which occur in many variations of molecular structure in a wide variety of Gram-negative bacteria. In Gram-positive bacteria, other compounds, such as peptides, regulate cellular activity and behavior by sensing the cell density. The degradation of the signaling molecule—called quorum quenching—is probably another important integral part in the complex quorum sensing circuit. Most interestingly, bacterial quorum sensing molecules also are recognized by eukaryotes that are colonized by QS-active bacteria. In this case, the cross-kingdom interaction can lead to specific adjustment and physiological adaptations in the colonized eukaryote. The responses are manifold, such as modifications of the defense system, modulation of the immune response, or changes in the hormonal status and growth responses. Thus, the interaction with the quorum sensing signaling molecules of bacteria can profoundly change the physiology of higher organisms too. Higher organisms are obligatorily associated with microbial communities, and these truly multi-organismic consortia, which are also called holobionts, can actually be steered via multiple interlinked signaling substances that originate not only from the host but also from the associated bacteria.

Keywords Quorum sensing · *N*-acyl homoserine lactones · Transkingdom signaling · Induced systemic resistance · Immunomodulation · Growth promotion

Introduction—Principles of Quorum Sensing

For a long time, it was considered that the most basic forms of life, single cell prokaryotic bacteria lacking a nucleus, are not able to develop a basic form of social behavior as a result of chemical communication among members of a population. Cooperative behavior by using autoinducer molecules was discovered first in bacteria that are living in symbiosis with a marine squid (Kaplan and Greenberg, 1985). The basic of this molecular communication, which is called "quorum sensing" (QS), and the signaling molecules involved (Waters and Bassler, 2005) were demonstrated via a very elementary experiment: by adding a so-called conditioned supernatant of a densely grown bacterial culture to a fresh, low cell density culture, the properties of the high density culture were conferred (Eberhard, 1972) (Table 1).

The marine bacterium *Vibrio fischeri* was the first bacterium to be studied for quorum sensing. As a signaling molecule,

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Table 1 Response of *Arabidopsis thaliana* towards HSL-molecules with different length side chains

HSL:	C6-HSL	3-oxo-C8-HSL	3-oxo-C10-HSL	3-oxo-C12-HSL	3-oxo-C14-HSL
Resistance	no effect	n.d.	n.d.	induced	strongly induced
Primary root elongation	induced	induced	reduced	no effect	no effect
Secondary root formation	no effect	no effect	induced	no effect	no effect
Adventitious roots	no effect	induced	strongly induced	induced	no effect
Root hair growth	no effect	no effect	induced	induced	no effect

Includes data from literature:

Ortiz-Castro et al. (2008), von Rad et al. (2008), Schikora et al. (2011), Bai et al. (2012), and Schenk et al. (2012)

N-(3-oxo)-hexanoyl-L-homoserine lactone (3oxoC6-HSL) was identified to control bioluminescence as an easily measurable outcome of cooperative behavior. The major biosynthetic enzyme (acyl homoserine lactone synthase - LuxI), uses S-adenosyl methionine and an acyl chain carrier protein to form the HSL-moiety. In each bacterial cell, the biosynthesis of HSL-molecules is working at a basal low level, and the HSL-compounds are being distributed not only inside the cells but also in the cell environment after diffusion through the membrane as a lipid-permeable amphophilic molecule. As a result, the HSL-concentration represents the cell density. When a certain cell density threshold ("quorum") is reached, HSL binds to a receptor protein (LuxR) in the bacterial cytoplasm (Fuqua et al., 1996). This HSL-receptor complex acts as a transcriptional regulator by binding to DNA promoter sequences (activating the expression of the *lux* operon and also other operons—see below). Thus, the response to the HSL-signaling compounds is an auto-inducing process, which makes this type of regulation extremely sensitive and economic (in terms of energy needed for the biosynthesis). Since the initial description of the luciferase operon in *V. fischeri*, genome sequencing analyses have revealed HSL-mediated QS homologues to LuxR/LuxI in many Gram-negative bacteria. Quorum sensing was found in bacteria living in association or symbiosis with higher organisms like plants and humans, as well as in so called free-living bacteria, where it plays a central role in biofilm formation. QS-regulated operons also are frequently found in plant or human pathogenic bacteria that harbor their virulence gene clusters under quorum sensing control (Eberl, 1999).

It has been argued, that "quorum sensing" faces evolutionary problems from non- or over-producing cheaters, which the non-cooperative diffusion sensing does not have (Hense et al., 2007). In a complex environment with many interacting biota and a complex physical structure of the microenvironment, the acquisition of indirect benefits (i.e., kin selection) through signaling has severe interference problems. However, considering bacterial cells living in a homogenous environment, as within a micro-colony and signaling among identical progenies, these evolutionary problems do not exist, because direct fitness benefits can be acquired. The diffusible auto-inducing signaling molecules are used to explore the environmental space in addition to scoring their own population density and the density of neighbor cells. "Speaking the same chemical language" improves the value of such a signaling tool, because it acquires additional integrated information about the overall habitat quality. Based on this information, the expression of the genetic potential can be optimized substantially. Therefore, the efficiency of genetic regulation, metabolic reactions, and organismic responses are substantially improved. Thus, the term "efficiency sensing", which has a direct positive impact on evolutionary selection, was suggested as alternative for "quorum sensing", because it also includes "diffusion sensing" (Hense et al., 2007).

Concerning the role of quorum sensing molecules in cross-kingdom interactions, it is not yet clear in every case, whether it is just a recognition of the chemical "smell" of a particular bacterial population by the interacting eukaryote, or if it also comprises a real signaling character (Cugini et al., 2008; Diggle et al., 2007; Stacy et al., 2012). Since quorum sensing is quite widespread in Gram-negative pathogenic bacteria, via e.g., 3-oxo-C12-homoserine lactone and similar molecules, these diffusible small molecules carry important information ("warning about potential pathogens") for the plant and could receive the term "cue", according to Stacy et al. (2012).

Some of quorum sensing active molecules also have non-signaling roles for important processes, such as nutrient scavenging, ultrastructure modification, and competition between bacteria (Schertzer et al., 2009). In particular, iron siderophores, like pyochelin and quinolone (see above), which interfere with cellular iron stores, are small signal molecules with central functions in the iron homoeostasis. All of these systems have in common, small diffusible molecules, which are used and diffuse out of the cell. Some even are actively transported. It recently has been suggested, that transport processes across the bacterial membrane may even improve the specificity of the different chemical forms of HSL-compounds (Minagawa et al., 2012).

Methods for Detection and Characterization of QS-Active Compounds

A precise, specific, and sensitive chemical analysis of quorum sensing autoinducer molecules was an essential prerequisite for innovative studies of quorum sensing related regulation in bacteria. By applying these techniques, a specific tracing of these QS-molecules in the environment and within eukaryotic hosts, colonized by HSL-producing bacteria, was made feasible (e.g., Götz et al., 2007).

In the case of quorum sensing molecules of the *N*-acyl homoserine lactone type, it proved fortunate for the development of research in this area that the first available chromatographic tools were soon assisted by highly sensitive and specific biosensors. These biosensors take advantage of the selective activation of promoters of HSL-regulated genes by autoinducer molecules. Different available operon fusion constructs of HSL-activated genes with the *lux*-casette, *gfp, rfp,* or *lacZ* have been reviewed by Fekete et al. (2010b). In addition, the quorum sensing regulated violacein production of *Chromobacterium violaceum* can be used successfully to indicate HSL-production or -degradation accordingly (McClean et al., 1997). These constructs also are available on plasmids, and can be transferred to different bacteria. However, HSL-biosensor bacteria must have their own HSL-production genes deleted or inactivated to avoid self-activation. The constructs usually have different specificities for either short or long side chain HSLs, but there are also reporter plasmids that allow detection of most HSLs with similar sensitivity (Thomson et al., 2000; Andersen et al., 2001). Nevertheless, one has to be careful in the application of these biosensors, because their "report" may be rather biased, and has to be supplemented with different means of chemical or immunological metabolite analyses. The occurrence of HSLs in certain habitats and their ecological significance has been stimulated by the use of green fluorescent protein (GFP) or red fluorescent protein/DsRed (RFP) fused to HSL-regulated promoters (Steidle et al., 2001). Concentrations of HSL down to 20 nmol L^{-1} can be detected by using these bioreporter constructs. However, this detection is quite selective, because, for example, in the case of the reporter strain *Pseudomonas putida* F117, the contained reporter plasmid pAS-C8 is 100 times more sensitive to 3-oxo-C12-HSL than C12-HSL (Steidle et al., 2001). Using these constructs, the *in situ* production of HSL-molecules can be, for example, detected on the surface of roots, thus resulting in the monitoring of "landscapes" of HSLs on colonized surfaces (Gantner et al., 2006). In microcolonies or polymer matrix embedded biofilms, where the diffusion is restricted, the local concentration of HSLs can reach high peak values. By using mathematical models for the calculation of the auto-regulated HSL-production in bacteria and restricted diffusion (Müller et al., 2006), local concentrations in the mmolL^{-1} range can be calculated, assuming just a volume of a 5-µm cube with enclosed *Burkholderia cepacia*. This phenomenon can have ecological relevance for interaction with eukaryotic hosts colonized by HSL-producing bacterial microcolonies or biofilms that could also contribute to counterbalance the possible degradation of HSL by quorum quenching reactions (see below).

Concerning chemical analysis, GC-based techniques of HSL-determination were developed first (Charlton et al., 2000). To increase the sensitivity of the method, for example, selective ion monitoring of the mass spectrometry (MS) detection or derivatization of the ß-oxo group to an oxime were applied (Charlton et al., 2000). As reviewed by Fekete et al. (2007), reversed-phase HPLC coupled with MS for selective detection has been applied in most cases (Morin et al., 2003). Frommberger et al. (2004) developed a microelectrospray interface to MS after nano-LC separation of the HSLs. Electrokinetic chromatography (MEKC) also has been applied successfully for the detection of HSLs and detection by MS (Frommberger et al., 2003). The most efficient separation of HSLs is with UPLC analysis, as described in detail by Li et al. (2006). The identification of enantiomers of HSLs in biological matrices also is possible by using an optimized GC-MS approach (Malik et al., 2009). The highest accuracy of molecular mass detection of HSLs has been achieved by using the positive ion Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) with mass errors of the peaks less than 0.1 ppm, as described by Fekete et al. (2007). However, even when using this highly resolving analytical tool, it is advisable to use two independent analytical approaches (e.g., UPLC and FTICR-MS) to unequivocally identify HSL-molecules, especially when the detection is from very complex matrices, such as nutrient broth medium, frequently used in microbiology.

Another independent method for the analysis of HSL molecules is based on immunochemistry. From several labs, monoclonal antibodies (MAB) have been produced against several HSL-molecules (Kaufmann et al., 2006, 2008; Chen et al., 2010a, b). These MAB not only allow the investigation of the biological influence of scavenging HSL (Park et al., 2007), but also the analysis of smaller sample sizes, and the localization of the distribution of HSL produced by bacteria associated with eukaryotes.

Diversity of Quorum Sensing Systems in Bacteria

In a variety of Gram-negative bacteria, biosynthesis of N-acyl homoserine lactones (HSL) occurs in many variations of the molecular structure. The spectrum of HSL-molecules ranges from short (C4-, C6-, and C8-) carbohydrate side chains to long (C12-, C14-, or even longer) side chains, and includes un-substituted as well as OH- and oxo-C3-substituted compounds. Although HSLs are the most common autoinducers in Gram-negative bacteria, structures like AI-2 (alternative autoinducer; furanosyl borate diester), AI3, quinolones (PQS), and a variety of other small molecules are known as signaling compounds (see Fig. 1, see also Effmert et al., 2012, this issue). In addition, lipid compounds, like *cis*-11-methyl-2-dodecenoic acid (also referred to as diffusible signal factor or DSF) (Wang et al., 2004) and 3-hydroxy palmitate methyl ester (3OH-PAME) (Flavier et al., 1997), have been identified as QS-mediating compounds. Furthermore, cyclic molecules, such as 2-heptyl-3-hydroxy-4-quinolone (PQS) and diketopiperazines (DKZ) also have been suggested as QS-signals of Pseudomonads (Holden et al., 1999; McKnight et al., 2000). In Gram-positive bacteria, a variety of partially cyclic peptides, AI-2 and butyrolactone (Folcher et al., 2001; Lyon and Novick, 2004) regulate cellular activity and behavior through sensing the cell density. AI-2 was proposed as a "universal" QS signal in bacteria, but this function is still questionable because it could just be an excreted end-product of a general metabolic pathway (Winzer et al., 2002).

Quorum Quenching—The Flip Side of the Coin

The degradation of HSLs was first found in bacteria, and is now documented for more than 20 genera (Uroz et al., 2009). Bacteria may even harbor several autoinducer modifying or degrading enzymes (Schipper et al., 2009). While *Pseudomonas aeruginosa* encodes for three functionally verified acylases (Huang et al., 2006; Soi et al., 2006), *Sinorhizobium* sp. NGR234 encodes for at least six functional proteins involved in autoinducer modification or degradation (Krysciak et al., 2011). These include three lactonases, one oxidoreductase, and two not further specified hydrolases. In *Pseudomonas putida*, IsoF, which is producing C10-HSL as major HSL-autoinducer, it has been demonstrated, that even during exponential growth, the HSLs are degraded to homoserines and other products (Fekete et al., 2010a). However, no known lactonase gene has been demonstrated in the genome of *P. putida* IsoF other then lactonase candidate genes. What the role of this rapid turnover of HSL in *P. putida* IsoF is remains to be investigated. One hypothesis is that exceeding levels of AHLs should be avoided, because otherwise the signal character of HSL-production is lost (Diggle et al., 2007). In addition, it may be of advantage to keep AHL levels under strict control in order to minimize the effects of the QS-compounds on the colonized plant, which could turn out to be of disadvantage to the bacteria because of the induction of defense reactions (see below). In some plants, like many legumes, the plant's HSL-hydrolyzing activities efficiently degrade HSLs and thus prohibit a substantial uptake of the

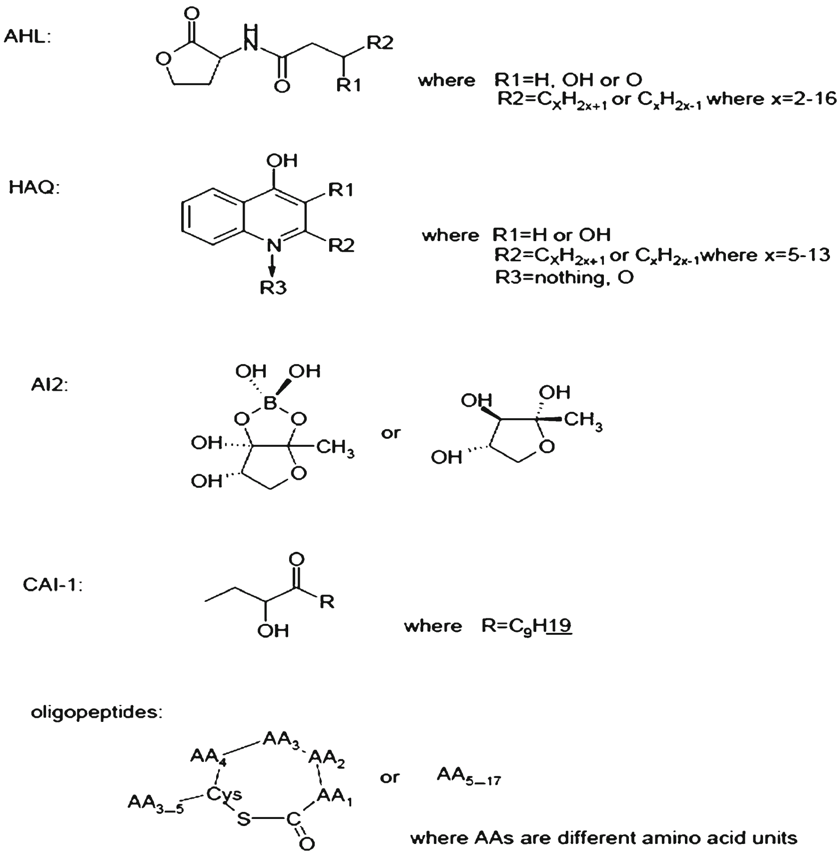

Fig. 1 Structures of quorum sensing molecules (examples): AHL: *N*-acyl homoserine lactones/HSL; HAQ: quinolone (PQS) of *P. aeruginosa*; AI2: autoinducer 2 (furanosyl borate diester); CAI-1: third autoinducer of *Vibrio harveij*

HSL-compounds (Delalande et al., 2005; Götz et al., 2007), while in many other plants, like barley and Arabidopsis, HSLs are rather stable in the rhizosphere and after uptake into the roots (Götz et al., 2007; von Rad et al., 2008).

HSL-degradation has been reported recently from a hyperthermophilic archaeon *Sulfolobus solfataricus* (Del Vecchio et al., 2009). The enzyme, named SsoPox, is bifunctional with an organophosphate hydrolase and N-acyl-homoserine lactonase activity. Since it has a broad specificity, it was selected for tests to be useful for disrupting quorum sensing in many bacterial species and applications. Quorum sensing/quenching was also proposed as new biofouling control paradigm in a membrane bioreactor for advanced wastewater treatment (Yeon et al., 2009).

Most interestingly, it has been demonstrated that a probiotic *Bacillus* isolate (QSI-1) from the intestine of the fish species *Carassius auratus gibelio* produces an enzyme that effectively degrades the HSL of the fish pathogen *Aeromonas hydrophila* YJ-1 (Chu et al., 2010). Furthermore, a thermostable *N*-acyl homoserine lactonase of a *Bacillus* sp. fed to fish effectively controlled the fish pathogen *Aeromonas hydrophila* (Cao et al., 2012). A significant reduction of HSL-related effects also was achieved by using monoclonal antibodies against HSL, as demonstrated by Kaufmann et al. (2008), who showed a reduction of cytotoxic effects of 3-oxo-C12-homoserine lactones on macrophages.

Many mycorrhizal and nonmycorrhizal fungi can degrade HSLs (Gonzalez and Keshavan, 2006). This is not surprising, since fungi always are in tight contact with heterotrophic bacterial communities that colonize hyphal surfaces (Mogge et al., 2000) or even live inside fungi (Frey-Klett et al., 2011). It is to be expected, that in the interaction of bacteria and fungi, quorum sensing compounds and quorum quenching activities by the fungal partner also are important. Features of the interaction in a di- or tripartite symbiosis, however, are not yet understood. The first evidence for quorum quenching in a diversity of root-associated fungi was obtained by Uroz and Heinonsalo (2008). Mammalian cells, like human epithelial cells in lungs and kidneys, are able to degrade HSLs efficiently. Cells with stronger exposition to bacterial populations, like lung epithelial cells, show a far higher ability for HSL-degradation. The aspect of perception and degradation of HSL-quorum sensing molecules was recently extensively reviewed by Teplitski et al. (2011).

Response of Eukaryotes to the QS-Compounds *N*-Acyl Homoserine Lactones (AHL)

Interactions with Fungi/Yeasts

Quorum sensing molecules of the HSL-type have been found to interact with fungi. Under the influence of e.g., 3-oxo-C8-HSL of *Pseudomonas aeruginosa* morphological changes of *Candida albicans* are induced (Hogan et al., 2004). It is to be expected, that many more of these interactions regularly occur in nature, because fungi are commonly colonized by bacteria. In some cases, even the occurrence of endofungal bacteria has been described. In the case of the plant growth promoting fungus *Piriformospora indica*, *Rhizobium radiobacter* was found to occur obligatorily as endofungal bacterium (Sharma et al., 2008), and this bacterium was shown to produce 3-oxo-C10 HSL as major autoinducer (D. Li and M. Rothballer, unpublished results). The role of autoinducers in this tight interaction has not at all been understood yet. New insight into the role of HSL-production of the bacterium in the plant growth promotion of the fungus/bacterium holobiont could be obtained by using QS-inhibitors, HSL analogues or HSL-negative bacterial mutants.

Interactions with Plants

In recent years, numerous lines of evidence have shown that plants also have evolved means to perceive and respond to quorum sensing compounds of the *N*-acyl homoserine lactone (HSL) type. Plants change their gene expression, alter their protein profile and modify their development, if HSLs are present in their surroundings (Bauer and Mathesius, 2004). More recent data demonstrate an impact of HSL-producing bacteria and HSLs itself on plants' defense system (Schuhegger et al., 2006). Several laboratories independently have reported the ability to modulate plant defenses by HSLs either produced by root-associated bacteria or added to the rhizosphere (plant nutrient solution in hydroponic systems) (Schuhegger et al., 2006; Schikora et al., 2011). Whether HSLs induce systemic responses or are transported within plants seems to depend on the structure of the HSL molecule, and in particular on the length and decoration of the fatty acid chain (see Fig. 1) (Schenk et al., 2012). In the interaction of bacteria with plants, other markers (so-called MAMPS) also need to be kept in mind, which have a high power as elicitors of plant defense responses (Boller and Felix, 2009). Therefore, HSL-compounds certainly constitute only a part of the molecular interaction pattern. Most clear-cut results of potential influences of single bacterial HSL-compounds have to be derived from axenic plant systems, when only the pure QS-molecule is applied in small amounts. The reports presented below summarize the current state of knowledge about the influence of bacterial *N*-acyl homoserine lactones on plants and their fate in the plant environment.

HSLs Modulate Plant Immunity

First indications that HSLs play a role in plant immunity originated from studies on the interaction of *Serratia liquefaciens* with tomato (Schuhegger et al., 2006). *Serratia* *liquefaciens* strain MG1 is known to produce C4- and C6-homoserine lactones. The *in situ* HSL-production on the root surface of these plants has been demonstrated using *in situ* HSL-reporter constructs (Gantner et al., 2006). It became apparent, that on the surface of plant roots a "landscape" of HSL-concentration is generated according to the colonization profile of HSL-producing bacteria. When *Serratia liquefaciens* MG1 was inoculated to roots of tomato (Microtom^R) plants, the systemic resistance against the fungal leaf pathogen *Alternaria alternata* was clearly increased (Schuhegger et al., 2006). Accordingly, the application of the HSL-negative mutant *S. liquefaciens* MG44 was much less effective in fungal biocontrol (Schuhegger et al., 2006). The induction of genes related to systemic pathogen response (e.g., chitinase, PR1) in tomato by C4- and C6-homoserine lactones in axenic test systems is contrasted by the response of *A. thaliana* towards these HSL-compounds with short side chains, because systemic resistance responses are not elevated (von Rad et al., 2008). Similarly, colonization with the HSL-producing *Serratia plymuthica* wild type protects cucumber plants from the damping-off disease caused by *Pythium aphanidermatum,* as well as tomato and bean from infection with the grey mold fungus *Botrytis cinerea* (Pang et al., 2009). This was corroborated by the finding that a *splI*$^-$ mutant of *S. plymuthica*, which is impaired in the production of HSLs, could not provide this protection (Pang et al., 2009).

Similarly, different HSLs have shown the ability to induce resistance against the bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*). *N*-3-oxo-tetradecanoyl-*L*-homoserine lactone (oxo-C14-HSL) significantly enhances the resistance against *Pst* in *Arabidopsis* plants (Schikora et al., 2011). Similar observations have been made in the case of biotrophic fungal pathogens. *Golovinomyces orontii,* the causal agent of powdery mildew in *Arabidopsis*, and *Blumeria graminis,* the causal agent of powdery mildew in barley, were blocked in attack when the plant roots were pre-treated with oxo-C14-HSL (Schikora et al., 2011). In addition, OH-C14-HSL and oxo-C12-HSL also have resistance-inducing potential, although weaker than C14-HSL derivatives. For example, many *Pseudomonas* and *Burkholderia* species characteristically produce those HSLs. In contrast, e.g., *Serratia liquefaciens* produces HSL with mostly short to medium length fatty acid side chains; no resistance induction was observed after pre-treatment of roots with C4- and C6- side chain HSLs (von Rad et al., 2008). The induction of genes related to systemic pathogen response, like chitinase and PR1, were not elevated implying no effect on resistance development. Therefore, it can be stated that fatty acid side chains of HSLs with different length provoke different reactions (see below).

Lasting induction of defense mechanisms leads to severe miss-regulation of plant metabolism and inhibition of plant growth and development. Therefore, defense mechanisms are tightly regulated and preferentially induced only after recognition of pathogens. The mitogen-activated protein kinase 3 and 6 (MPK3 and MPK6) activities, both known to be involved in plant defense mechanisms are key elements in this regulatory network. In naive plants, treatment with the bacterial Pathogen Associated Molecular Pattern (PAMP) flg22 triggers a transient activation of MPK3 and MPK6; this activity normally decreases 30 min after treatment to its original level (Boller and Felix, 2009). However, in plants pretreated with HSL a prolonged activation of MPK3 and MPK6 has been observed (Schikora et al., 2011). The prolonged activation of MPK6 in HSL-pretreated plants is followed by stronger induction of several defense-related genes e.g., *WRKY22* and *WRKY29*, as well as the defensin *Pdf1.2.* Whether this modified expression represents only a part of the HSL-induced resistance remains to be verified.

HSL-Dependent Modulation of Plant's Development

Another, not less-interesting interaction between plants and HSLs is the impact that *N*-acyl homoserine lactones have on plants' development. Mathesius et al. (2003) showed that upon treatment with different HSLs, one third of the differentially accumulated proteins are specific for the respective HSL (Mathesius et al., 2003).

Numerous reports presented altered root development in plants (mostly *Arabidopsis*) exposed to different HSL molecules. As indicated above, *N*-acyl homoserine lactones vary in the length of the lipid chain and the substitution on the C3 fatty acid carbon residue. As shown by von Rad et al. (2008), treatment of roots with 1–10 μM C4- and C6-HSL clearly promoted root growth. At these concentrations of HSL-treatment, the ratio of auxin/cytosine was increased, which explains the observed phenotypic effects. Therefore, the character of plant response is dependent on the different structural details of the HSL moiety (Schenk et al., 2012). Ortiz-Castro et al. (2008) have shown that the application of C10- and C12-HSL strongly induced shortening and thickening of the primary root and root hair formation. Recently, Bai et al. (2012) reported that oxo-C10-HSL, but not its unmodified homologue C10-HSL, induces the formation of adventitious roots in Mung bean plants (*Vigna radiata*). The authors suggest that oxo-C10-HSL accelerates the basipetal auxin transport and that the auxin-dependent formation of adventitious roots relies on H_2O_2 and NO-dependent cGMP signaling (Bai et al., 2012).

Fate of Different HSL and Systemic Responses in Plants

An important question is whether the transport of HSLs within the plant is a prerequisite for their impact on plants. The transport of HSLs within plants has been an issue in several reports. HSL-compounds with acyl side chain lengths smaller than C8 have been found to enter the roots more readily and are transported up to the shoots (Götz et al., 2007). This analysis was made possible by applying highly resolving and sensitive fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) and ultra-high performing liquid chromatography (UPLC) (Fekete et al., 2010b and see former section). Additionally, it was shown that the ^{3}H-labelled C6- and C8-HSLs are taken up into the central cylinder, and that their transport within the roots is an active process, which is further accelerated by the transpiration flow (T. Riedel, unpublished results). HSL-compounds with long side chains (longer than C10) stick to the root surface and are not transported substantially within barley, maize, or *Arabidopsis* (Götz et al., 2007; von Rad et al., 2008; Schikora et al., 2011). Hence, the correlation between ability for translocation and growth promotion is still elusive.

Systemically induced resistance is yet another aspect of HSLs' influence on plants. Although oxo-C14-HSL could not be detected in shoots, pretreatment with oxo-C14-HSL induced resistance in leaves against biotrophic leaf pathogens, consistent with the concept of systemic disease resistance (Schikora et al., 2011). To uncover the nature of an HSL-induced signal will be an important step in the understanding of the mechanism by which homoserine lactones influence host plants. While Schikora et al. (2011) identified MPK6 as essential kinase in the oxo-C14-HSL signaling, Liu et al. (2012) dicovered that the GCR1 G-protein and the canonical Gα subunit GAP1 are required for C6-HSL signaling in *Arabidopsis*.

The interaction between anti-microbial and anti-herbivore defense in plants can be regarded as another example of inter-kingdom crosstalk. When herbivore resistance in *Nicotiana attenuata* was tested using larvae of *Manduca sexta* in the presence or absence of the QS-compound C6-HSL, there was a 4-fold mass gain of the larvae in C6-HSL treated plants (Heidel et al., 2010). Microarray analysis of the plants elicited with C6-HSL and jasmonic acid-inducing fatty acid-amino acid conjugates revealed a down-regulation of a proteinase inhibitor. The results suggest that the increased performance of *M. sexta* was due to direct or indirect effects of C6-HSL on JA-mediated defense (Heidel et al., 2010).

A variety of plants and algae produce mimic substances for bacterial autoinducers, as reviewed by Bauer and Mathesius (2004) and Gonzalez and Keshavan (2006). These compounds have been shown to interfere efficiently with the quorum sensing system of bacteria (pathogens or saprothrophs) that colonize the root and shoot of plants. Transgenic plants that harbor the synthesis gene for *N*-acyl homoserine lactones turned out to be able to deregulate the HSL-production of pathogenic bacteria, and thus successfully reduced their virulence. In addition, transgenic tomato plants, producing different types of HSL-compounds, altered the activity of plant growth promoting bacteria in the rhizosphere (Barriuso et al., 2008).

Interaction with Animals/Human

Long side chain as well as short side chain *N*-acyl homoserine lactones have been found to modulate the host immune response and inflammatory signaling pathways of invertebrates (extensively reviewed by Cooley et al., 2008; Teplitski et al., 2011). For mammalian systems, the effects of 3-oxo-12-HSL, the major autoinducer of the pathogenic bacterium *Pseudomonas aeruginosa* has been thoroughly investigated, because lung infections, especially in immunocompromised and COPD-patients, are often very severe or even lethal. The *P. aeruginosa* HSL interacts with the host's immune response via a mechanism independent of the canonical pathogen-associated molecular pattern recognition receptor signaling pathway (Kravchenko et al., 2006). It has been demonstrated, that IL-8 is induced in epithelial cells and human lung fibroblasts (Smith et al., 2001), neutrophils are attracted (Zimmermann et al., 2006), and T-cell differentiation and cytokine production are inhibited (Ritchie et al., 2007). The expression of CD86 by LPS-stimulated human blood isolated dendritic cells also was inhibited by 3-oxo-C12-HSL (Boontham et al., 2008). For the immuno-modulatory activity of HSL, a chain length greater than 10 C-atoms, an intact homoserine lactone ring, and oxo- or hydroxyl substitutes are important prerequisites. It seems that the primary immune response, triggered by microbial elicitors like bacterial lipopolysaccharide, was halted or neutralized by the interaction with low, non-toxic concentrations of 3-oxo-C12-HSL (R. Bernatowicz, T. Binder, and S. Krauss-Etschmann, unpublished results).

Summary

The discovery of the general importance of autoinducer signaling molecules involved in quorum or efficiency sensing of bacteria and the dynamic and diverse responses by eukaryotes towards this apparent key process of organisation of strategic bacterial population behavior has opened the new field of sociobiology for prokaryotic singular cells. The regulation of synthesis and degradation of these diffusible signaling or "scout" molecules is still not completely understood. Especially the role of the diverse degradation and modification reactions, which are found even in some producer strains as well as in accompanying prokaryotic and eukaryotic organisms, awaits further elucidation. Apparently, these QS-modifying activities are part of a finely tuned optimization strategy, which may result as an efficient "hiding" mechanism on the bacterial side and as a defense strategy on the eukaryotic side. It is quite possible, that

QS-signaling of bacteria has contributed substantially to establishing symbiotic interactions in some cross-kingdom interactive biological units, similar to those in the interaction of rhizobia symbionts with their specific legume hosts. The concept of holobiontic or hologenomic systems (Zilber-Rosenberg and Rosenberg, 2008) matches well with the findings of multiple cross-kingdom signaling molecules and events (Teplitski et al., 2011). Furthermore, it is speculated that these basic discoveries about bacterial auto-inducer systems may provide a new and innovative way to fight back attacks by plant and human pathogenic bacteria with much reduced risk of the development of resistance development (Park et al., 2007). The opportunity is seen in the fact that treatment with "anti-QS drugs" is not aiming at directly killing the bacterial enemy, but rather spoiling its strategies of attack. This weakening of the pathogen attack may give the natural immune defense of the patient or additional therapies important advantages to eliminate the pathogen. However, future pioneer trials have to demonstrate, whether this keen concept is really turning to a fruitful and widely applicable practice.

Soils, especially rhizosphere soil (Elasri et al., 2001; Riaz et al., 2008; Effmert et al., 2012, this issue) can be regarded as a reservoir of a high diversity of QS-active microbes and HSL-production of Gram-negative bacteria in particular. In addition, a rich pool of diverse HSL-degrading microbes also can be expected in the rhizosphere. Thus, this natural hotspot of QS could be further exploited to achieve new possibly effective biotechnological approaches to control crop pathogenic bacteria and to support crop growth.

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REVIEW ARTICLE

Microbes as Targets and Mediators of Allelopathy in Plants

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Abstract Studies of allelopathy in terrestrial systems have experienced tremendous growth as interest has risen in describing biochemical mechanisms responsible for structuring plant communities, determining agricultural and forest productivity, and explaining invasive behaviors in introduced organisms. While early criticisms of allelopathy involved issues with allelochemical production, stability, and degradation in soils, an understanding of the chemical ecology of soils and its microbial inhabitants has been increasingly incorporated in studies of allelopathy, and recognized as an essential predictor of the outcome of allelopathic interactions between plants. Microbes can mediate interactions in a number of ways with both positive and negative outcomes for surrounding plants and plant communities. In this review, we examine cases where soil microbes are the target of allelopathic plants leading to indirect effects on competing plants, provide examples where microbes play either a protective effect on plants against allelopathic competitors or enhance allelopathic effects, and we provide examples where soil microbial communities have changed through time in response to allelopathic plants with known or potential effects on plant communities. We focus primarily on interactions involving wild plants in natural systems, using case studies of some of the world's most notorious invasive plants, but we also provide selected examples from agriculturally managed systems. Allelopathic interactions between plants cannot be fully understood without considering microbial participants, and we conclude with suggestions for future research.

Keywords *Alliaria petiolata* · Glucosinolates · Microbial degradation · Mycorrhizae · Phenolics · Rhizobia · Invasive plants

Allelopathy and Soil Microbes

Allelopathy, generally, is considered as a form of negative chemical communication between organisms, whereby one participant (the donor) in an interaction produces a compound(s) that is released in the environment in ecologically relevant quantities that negatively impacts the fitness of other participants (the receivers); the effect presumably benefits fitness of the donor. While the concept of allelopathy extends back to at least Theophrastus in the third century B.C., who invoked this phenomenon as an explanatory mechanism of plant growth, abundance, or community structure in natural systems, the concept has fluctuated in popularity over time (see Willis, 2007 for review). Allelopathy often has been subjected to criticisms of ecological relevance that other phenomena, such as resource competition, have not, thus explaining why it has fallen out of favor during certain time periods. However, studies of allelopathy in terrestrial systems have experienced a tremendous "rebirth" in the last 20 years as interest has risen in describing biochemical mechanisms responsible for structuring plant communities, determining agricultural and forest productivity, and explaining invasive behaviors in introduced organisms. More rigorous observational and experimental approaches, along with better analytical techniques, have

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been brought to bear on this issue yielding better data. As a result, allelopathy as a significant ecological phenomenon has now become firmly entrenched in the literature, with entire books and journals devoted to the topic (e.g., Reigosa et al., 2006).

In terrestrial systems, much emphasis has been placed on allelopathic interactions that have focused on direct effects (e.g., toxicity) of putative allelochemicals on plant growth. In addition to agriculturally-focused studies with plants like wheat, rye, and sorghum (e.g., Belz, 2007), one of the more famous examples of a wild plant with direct allelopathic effects is walnut, *Juglans nigra*, which produces the allelopathic compound juglone (Jose, 2002). This compound is released into the soil in measureable quantities and is believed to be largely responsible for the depauperate plant community around walnut trees. The emphasis of many recent studies has been on allelopathic invasive plants, including the herbs *Centaurea stoebe* (aka *C. maculosa*) (Callaway and Ridenour, 2004), *Solidago canadensis* (Abhilasha et al., 2008), and *Alliaria petiolata* (Prati and Bossdorf, 2004), the shrubs *Lonicera maackii* (Dorning and Cipollini, 2006) and *Artemisia* spp. (Lydon et al., 1997), and the trees *Ailanthus altissima* (Small et al., 2010) and *Eucalyptus* spp. (Sasikumar et al., 2001). While debate continues for some of these species (e.g., Duke et al., 2009; Bais and Kaushik, 2010), field or laboratory studies that use growing plants, field- or laboratory-conditioned soils, and tissue extracts have revealed that these plants and the compounds that they produce can have direct biochemical effects on other plants in some situations. Early criticisms of the ecological relevance of allelopathy, however, involving issues with allelochemical production, stability, and degradation in soils, lead to the contention that allelochemicals rarely reach concentrations with meaningful direct effects in the field due to microbial degradation (Willis, 2007). To understand these dynamics, an understanding of the chemical ecology of soils and the organisms it contains is of paramount importance (Romeo, 2000; Inderjit, 2005). This concern has been increasingly incorporated in studies of allelopathy (Kaur et al., 2009), and is being increasingly appreciated as an essential predictor of the outcome of allelopathic interactions.

The soil microbial community is diverse, and its composition varies greatly in space and time. Among the more important types of soil biota with relevance to allelopathy are the many free-living and symbiotic bacteria and fungi that are found in the plant rhizo- and mycorrhizosphere (Johansson et al., 2004, Fig. 1). The presence of a live soil microbial community can greatly modify allelopathic effects of some plants, and sometimes beneficial microbes themselves appear to be directly negatively affected by allelopathic compounds (Table 1). It is widely known that plant species will culture somewhat specific microbial populations in their rhizospheres that have subsequent feedbacks on conspecific and heterospecific individuals grown in the same soil. This effect can be due to such factors as the amount and form of carbon and other nutrients that the plant provides to the soil, but is also due to allelochemicals with direct positive and negative effects on microbes (Reinhardt and Callaway, 2006). In this review, we examine cases where microbes are directly affected by allelopathic plants leading to indirect effects on competing plants, provide examples where microbes either protect plants from allelopathic competitors or enhance allelopathic effects, and provide examples where soil microbial communities have changed through time in response to allelopathic plants with potential effects on plant communities. We focus primarily on interactions involving wild plants in natural systems, exploiting the emerging literature on invasive plants, while providing selected examples from studies on agricultural plants in managed systems. We use the term “microbe” throughout to refer to both bacteria and fungi.

Allelopathic Effects on Beneficial Microbes

From an ecological and evolutionary perspective, allelopathic effects of plants on soil microbes may have indirect effects on competing plants that are just as important as direct effects. Such effects on soil microbes could arise from direct selection for this effect, or could be a side-effect of allelochemicals selected for their phytotoxic effects. Either way, effects on the microbial community on which competing plants rely for nutrient and water uptake, nutrient cycling, and other services, could promote fitness of an allelopathic plant as long as it does not harm itself in the process. This possibility is exemplified by allelopathic plants that inhibit mutualistic bacteria or fungi that other plants require for optimal growth, but that the donor plant does not require. It is also likely most apparent in environments lacking an evolutionary history with the allelopathic plant and the allelochemicals that it produces (Callaway and Ridenour, 2004).

Mycorrhizal Fungi as Targets of Allelopathic Effects One well-studied example of the effect of an allelopathic plant on mutualistic microbes is that of the widespread Eurasian invader, *Alliaria petiolata* (*Alliaria*), on arbuscular and ectomycorrhizal fungi (AMF and EMF; Fig. 1). This plant belongs to the family (Brassicaceae) well-known for its lack of association with mycorrhizal fungi (Shreiner and Koide, 1993). This may contribute to the ability of plants from this family to be rapid colonizers of disturbed habitats worldwide where depauperate mycorrhizal communities could limit colonization by mycorrhizal-dependent plants. However, it also permits plants from this family to inhibit mycorrhizal fungi of neighboring plants without the possibility

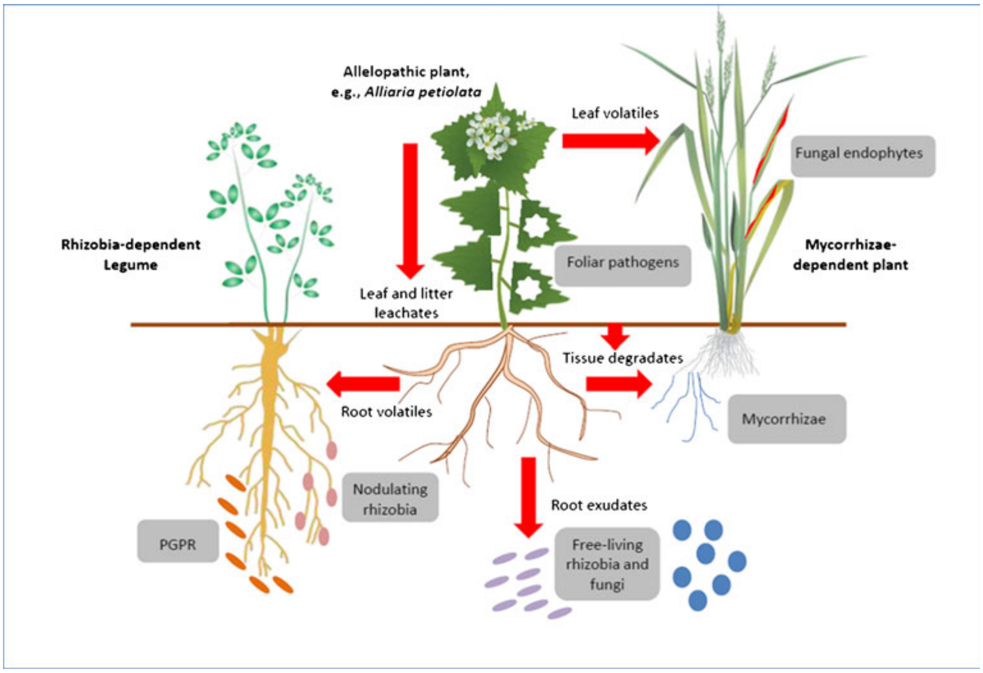

Fig. 1 Sources of alleochemical inputs and routes of transport (*red arrows*) and microbes that are targets and mediators of allelopathy in plants (*grey boxes*)

of harming themselves in the process, which might provide them with a competitive advantage. Studies of the biochemical basis of the effect have focused historically on the glucosinolates, a class of compounds produced by Brassicaceous plants that have been proposed as a mechanism of resistance to either pathogenic or beneficial fungi (Shreiner and Koide, 1993). Vaughn and Berhow (1999) first raised the possibility that allyl isothiocyanate (AITC) and benzyl isothiocyanate (BzITC), toxic degradation products of the two major glucosinolates that *Alliaria* produces, sinigrin and glucotropaeolin, respectively, could have effects on mycorrhizal fungi. Roberts and Anderson (2001) revealed this potential when they showed that aqueous leaf extracts of *Alliaria* could inhibit spore germination of AMF in addition to having some direct effects on plant performance in laboratory studies. *Alliaria* density in the field also negatively correlated to mycorrhizal inoculum potential of field soils. Stinson et al. (2006) followed these studies by showing that tree seedlings exposed to aqueous *Alliaria* extracts or *Alliaria*-conditioned soils had reduced AMF infection rates, with indirect negative effects on growth. Wolfe et al. (2008) later showed similar effects of *Alliaria* on EMF of pine trees in the field. Callaway et al. (2008) demonstrated that the extent of allelopathic effects of *Alliaria* depended on the degree of mycorrhizal dependence of target plant species, and that the allelopathic effects of *Alliaria* on AMF spore germination and infection rates had a biogeographical basis. Specifically, AMF from soils lacking an evolutionary history with *Alliaria* were more susceptible to allelopathic effects of its extracts, supporting predictions of the “Novel Weapons Hypothesis” (Callaway and Ridenour, 2004). Moreover, they showed that both glucosinolate and flavonoid-enriched extracts of *Alliaria* leaves were partly responsible for allelopathic effects on AMF spores, and that this mixture worked synergistically to inhibit spore germination in *Alliaria*-naïve soils. Koch et al. (2011) later showed that this combination of allelochemicals from leaves could inhibit colony growth of a single AMF species *in vitro*. Lankau et al. (2009) showed that allelopathic potential of *Alliaria* appeared to decline with the age of the population, which correlated with declining root glucosinolate levels with population age. In turn, Barto et al. (2012) showed that AMF infection rates of sugar maple were reduced and AMF community composition was affected by the presence of *Alliaria* in the field, an effect most noticeable in an area with a presumably younger population of *Alliaria*. Lankau (2011a) showed that effects of *Alliaria* populations on mycorrhizal richness and community structure in the rhizosphere of *Quercus rubra* were correlated with concentrations of glucosinolates and the hydroxynitrile glucoside, alliarinoside, in their roots. Importantly, as *Alliaria* pushed mycorrhizal community structure increasingly away from the native condition, growth of *Q. rubra* and another native tree increasingly declined. However, correlations with *Alliaria* population age were not as apparent.

While studies like Callaway et al. (2008) attempted to expose AMF to ecologically realistic concentrations of putative allelochemicals from *Alliaria* through soil conditioning or extract dilution, one missing link from these studies was the assessment of field concentrations of putative allelochemicals. Barto and Cipollini (2009b) were unable to extract glucosinolates from field soils under *Alliaria*, but did detect some potentially bioactive flavonoid derivatives through biomimetic extraction that were related to those that showed negative effects on AMF. However, half lives of

Table 1 Examples of cases where microbes of different taxonomic groups are the target of allelopathic effects, experience community shifts, and degrade or enhance allelopathic effects of plants

Plants involved	Type of microbe	Identity	Reference
Targets of Allelopathy			
Centaurea stoebe	PGPR[a]	mixed[b]	Pollock et al., 2011
Polygynum avuncular	PGPR	*Azotobacter* spp.	Alsaadawi and Rice, 1982
Polygynum avuncular	Rhizobacteria	*Rhizobium* spp.	Alsaadawi and Rice, 1982
Several weed species	Rhizobacteria	*Bradyrhizaobium japonicum*	Mallik and Tesfai, 1988
Sysimbrium loeselii	AMF[c]	mixed	Bainard et al., 2009
Alliaria petiolata	AMF, EMF[d]	mixed; *Glomus intraradices*	e.g., Stinson et al., 2006; Wolfe et al., 2008; Callaway et al., 2008; Koch et al., 2011
Brassica nigra	AMF	mixed	Lankau et al., 2011
Allelochemicals Cause Microbial Community Shifts			
Alliaria petiolata	AMF	mixed	Barto et al., 2012
Alliaria petiolata	AMF and soil bacteria	mixed	Lankau, 2011b
Cunninghamia lanceolota	Pathogenic fungi	mixed	Zhang, 1997
Oryza sativa	mixed	mixed	Kong et al., 2008
Degraders of Allelopathic Compounds			
Cucumis sativus	Fungus	*Trichoderma harzianum* SQR-T037	Chen et al., 2011
Bambusa chungii, Pinus massoniana, Oryza sativa	Gram negative bacteria	*Pseudomonas putida* 4CD1 *Pseudomonas nitroreducens*	Zhang et al., 2010
		Pseudomonas putida 4CD3	
	Fungus	*Rhodotorula glutinis*	
Enhancers of Allelopathy			
Secale cereale	Gram negative bacteria	*Actinetobacter calcoaceticus*	Chase et al. 1991
Herbaceous Plants	AMF CMN[e]	mixed	Barto et al., 2011
Festuca rubra	Endophytic fungi	*Epichloe festucae*	Vásquez-de-aldana et al., 2011
Lolium arundinaceum	Endophytic fungi	*Neotyphodium coenophialum*	Rudgers and Orr, 2009

[a] *PGPR* plant growth promoting rhizobacteria, [b] *mixed* mixture of species, [c] *AMF* arbuscular mycorrhizal fungi, [d] *EMF* ectomycorrhizal fungi, [e] *CMN* common mycorrhizal network

most of these compounds were exceedingly short in non-sterile field soils. In contrast, Cantor et al. (2011) were able to detect AITC in field soils at levels that were sufficient to inhibit a single AMF species in laboratory bioassays. This was an important find, as *Alliaria* produces lower levels of glucosinolates than many of its weedy relatives, and levels also vary in concentration in leaves and roots through the season (Vaughn and Berhow, 1999). If glucosinolates or their degradation products are partly responsible for its allelopathic effects, then they must generally work in low concentrations and would be expected to vary in importance throughout the season.

Despite the evidence that *Alliaria* or its extracts can affect AMF spore germination, growth, infection rates, and community structure, some studies have not found major allelopathic effects of *Alliaria* on these variables. For example, Burke (2008) found little effect of *Alliaria* presence on either AMF infection rates of three forest herbs or AMF community structure in a field study. Barto and Cipollini (2009a) and Barto et al. (2010a) showed direct effects of *Alliaria* extracts on germination and growth of *Impatiens pallida* in pots or in glass chambers, but no effect on AMF infection rates if the *I. pallida* plants were colonized before exposure to *Alliaria* extracts (Barto et al., 2010a). Despite finding direct effects of *Alliaria* extracts on AMF colony growth, Koch et al. (2011) found little effect of *Alliaria* on AMF species richness or community structure in a pot study with field soils. Lankau (2011b) showed that effects of *Alliaria* on AMF community composition in the field appear to change with the age of *Alliaria* populations, showing declines in AMF species richness and shifts in community structure as you move from young to medium-aged populations, followed by a rebound in richness and in the number of *Alliaria*-sensitive AMF species in older populations of *Alliaria*. Thus, different conclusions about the effect of *Alliaria* on mycorrhizae and subsequent indirect effects on plant competitors can be reached at different field sites that could be related to variation in “toxicity” of particular *Alliaria* populations (Lankau, 2011b).

From these studies, ecologically important effects of *Alliaria* on AMF (and possibly EMF) in the field seem clearly possible, but the magnitude of these effects depends on plant density, age, and allelopathic potential of the *Alliaria* population, the evolutionary history of the soils and its AMF community with *Alliaria*, the dependence of target plants on AMF, and the timing of exposure to *Alliaria* during the development of mycorrhizal symbioses. While declines in AMF infection rates of native species would seemingly have negative fitness effects, it is not always clear that changes in AMF community composition, when seen, will necessarily have negative effects on competing plants (but see Lankau, 2011a). Providing additional support for potentially important allelopathic effects are reports of the impact of other weedy Brassicaceous species on mycorrhizae, including that of *Brassica nigra* (Lankau et al., 2011) and *Sysimbrium loeselii* (Bainard et al., 2009), but none of these cases have been as fully developed as that of *Alliaria*, and may be subject to the same limitations that intensive study of this species has revealed. The role of glucosinolates in effects of Brassicaceous species on mycorrhizae also remains to be fully elucidated. By taking advantage of several glucosinolate-containing, but mycorrhizal species, along with several non-mycorrhizal Brassicaceous species, Vierheilig et al. (2000) found that gluconasturtin (2-phenylethylglucosinolate) was the only glucosinolate that could consistently be associated with non-mycorrhizal status in these plants. Glucotropaeolin, a major glucosinolate in *Alliaria*, was found in mycorrhizal species, was induced to increase in both non-hosts and hosts by mycorrhizal inoculation, and variation in its concentration in roots was unrelated to variation in mycorrhizal status (Ludwig-Muller et al., 2002). Since *Alliaria* produces insignificant quantities of gluconasturtin, if any, that seems to leave sinigrin and AITC as the leading candidates if glucosinolates are involved in allelopathic suppression of AMF by *Alliaria*. However, a mechanism involving other chemical weapons of *Alliaria* could be important, including flavonoids and their glycoside derivatives, alliarinoside and other hydroxynitrile glucosides, and cyanide (Callaway et al., 2008; Barto et al., 2010b; Lankau, 2011b; Frisch and Moller, 2012). Phenolics common in litter and humus, for example, have been shown to have both direct effects on plants and to affect mycorrhizal physiology (e.g., Boufalis and Pellisier, 1994). While certain flavonoids can be stimulatory to AMF, some isolated flavonoids that *Alliaria* contains, such as apigenin, inhibit AMF spore germination and hyphal growth (Becard et al., 1992), in addition to having some direct phytotoxic effects (Cipollini et al., 2008). When it occurs, allelopathic inhibition of mycorrhizae is likely multifaceted, and is not yet fully understood even in a system as well studied as *Alliaria*.

Beneficial Bacteria as Targets of Allelopathy Other types of mutualistic microbes, including free living and symbiotic rhizobial bacteria, can differentially benefit plants and be affected by allelopathic plants (Fig. 1). As for mycorrhizal fungi, the ability to inhibit symbiotic rhizobia could provide allelopathic plants an advantage when in competition with plants that benefit strongly from these mutualists. This possibility has been studied extensively in weedy plants of agricultural systems because of the importance of nitrogen-fixing nodulating rhizobia to the success of Leguminous crop species. Using one such system, Mallik and Tesfai (1988) showed that shoot extracts of several weed species, including *Chenopodium album*, *Cyperus esculentus*, and *Helianthus annuus*, not only had direct effects on soybean (*Glycine max*) seed germination and seedling growth, but also severely reduced or eliminated nodulation by *Bradyrhizobium japonicum*. Effects of extracts were dose dependent, however, being stimulatory at lower concentrations in some cases. In that study, *Polygynum pennsylvanicum* was found to have little effect on *B. japonicum*, but Alsaadawi and Rice (1982) found that extracts containing phenolic glycosides of *Polygynum avuncular* had direct effects on germination and growth of *Chenopodium album*, and inhibited some nitrogen-fixing strains of *Rhizobium* in both the lab and the field. Sasikumar et al. (2001) showed that phenolic-containing extracts of several *Eucalyptus* species used in agroforestry inhibited seed germination and growth of *Cajanus cajan*, and could also inhibit nitrogenase activity in already nodulated roots of this legume. In a rare study on a wild system, Larson and Schwartz (1980) exposed black locust (*Robinia pseudoacacia*) and red clover (*Trifolium pratense*) seedlings to litter from several old field species, including *Solidago altissima*, and found that nodulation and nitrogen fixation rates generally declined at high litter exposures in parallel with declines in growth of these species. On the other hand, *R. pseudoacacia* (like some other legumes) has been examined for its own direct allelopathic effects (Nasir et al., 2005) and for its ability to associate with a wide variety of nodulating rhizobia across its native and invasive range (Callaway et al., 2011). This suggests that some nodulating bacteria are susceptible to allelochemicals from some plants, but either evade or suppress those of its host. This appears to be true for mutualistic fungi, like *Piriformaspora indica*, that act as beneficial endophytes in roots of a wide variety of plants including those of the Brassicaceae that do not form mycorrhizal associations (Jacobs et al., 2011). In summary, while the potential ecological effects of a variety of weedy plants on nodulating rhizobia have been documented, most of the studies have involved plants in managed systems. No cases of the inhibition of nodulating rhizobia by allelopathic plants have been as thoroughly examined as the interaction of some allelopathic plants, like *Alliaria*, with mycorrhizae.

Other beneficial soil bacteria, like the Plant Growth Promoting Rhizobacteria (PGPR), also may be affected by allelopathic plants (Fig. 1). The PGPRs include strains of many species of free-living bacteria, such as *Pseudomonas fluorescens, Bacillus subtilis*, and other species, that associate with the roots of plants providing benefits to some of them in the form of enhanced growth and disease resistance (Kloepper et al., 2004). Although much less studied in this regard than nodulating rhizobia, Alsaadawi and Rice (1982) found that extracts containing phenolic glycosides of *Polygynum avuncular* that affected nodulating *Rhizobium* strains could also negatively affect free living nitrogen fixing strains of *Azotobacter*, a PGPR. A range of free living bacterial species, some of them possibly PGPRs, were inhibited at both the population and community level by (±) catechin, a putative allelochemical from *Centaurea stoebe* (Pollock et al., 2011). In some cases involving free living bacteria, allelopathic effects have been reported at the community or ecosystem level, with often unknown consequences. Like its effect on mycorrhizal species richness and community structure, Lankau (2011a, b) showed that species richness and structure of the bacterial communities associated with *Alliaria* populations varied with the age and allelopathic potential of the invasive population, with unknown consequences for competing plant performance. Often, a known microbially-associated ecological function, like nitrogen mineralization rate or decomposition rate, has been shown to respond to an allelopathic plant without an examination of the microbial community itself (Ehrenfeld, 2003). Even some microfauna with important ecosystem functions can be affected by allelochemicals. When incorporated into soils, benzyl isothiocyanate (BzITC) from Brassicaceous cover crops not only has a pest and pathogen-resistance function (Brown and Morra, 1997), but also deleteriously affects the springtail (*Folsomia fimetaria*), a beneficial soil-dwelling micro-arthropod that plays a significant role in soil organic carbon and nutrient cycling (Jensen et al., 2010). For free-living bacteria and other soil biota that perform important ecological functions and lack a tight association with particular species, however, it is more difficult to argue that negative allelopathic effects on them could occur without harm to the allelopathic plant itself. Species vary, however, in their response to PGPRs and other organisms, including, presumably, the allelopathic plants themselves. Furthermore, some species of free living bacteria that have growth-promoting properties can also exhibit allelopathic effects (Barazani and Friedman, 2001), thus indicating why it is sometimes difficult to determine the identity of the allelochemical donor in allelopathic interactions.

In summary, evidence is accumulating that microbial mutualists in soils, such as mycorrhizal fungi or nodulating bacteria, can be the target of allelopathic effects by plants. Such effects may give allelopathic plants a competitive advantage when competing with sensitive species, especially those highly dependent on symbiotic microbes. While free living microbes that confer either fitness-enhancing effects on individual plants or play broader ecological roles also may be subjected to allelopathic effects, these effects are less likely to be the result of specific targeting, and are less clearly beneficial for the allelopathic plant that would also benefit from ecosystem services of such microbes.

Microbial Protection from Allelopathy

Microbial Degradation of Allelochemicals Microbes play an important role in limiting allelopathic effects in natural environments, as has been demonstrated in numerous studies that compare effects in sterile and non-sterile environments. One of the first demonstrations of this phenomenon used an indirect approach, incubating leaves of *Gmelina arborea* for varying times before adding corn seeds to conduct a germination bioassay (Hauser, 1993). When microbial degradation of leaf material was allowed to occur for 14 days before bioassays took place, the germination rates were significantly higher, suggesting that microbes degraded the allelopathic compounds. More direct demonstrations followed with Heisey (1996) showing that ailanthone from *Ailanthus altissima* inhibited cress radicle growth more in sterile soil than in non-sterile soil. More recent work has demonstrated this phenomenon for other trees, as well as herbaceous plants and grasses producing a range of allelopathic compounds (Fernandez et al., 2008; Kaur et al., 2009; Rudgers and Orr, 2009; Zhu et al., 2011). Mechanisms by which microbes reduce allelopathic effects include degradation of allelochemicals, increasing tolerance of target plants to allelopathic effects, and altering phytochemical profiles of allelopathic plants to reduce production of allelochemicals.

Microbial degradation is the reason most often given to explain microbial reductions of allelopathic effects, and it has been demonstrated in studies that have found increased growth of bioassay plants along with reduced recovery of allelochemicals under non-sterile conditions (Inderjit and Foy, 1999; Chiapusio and Pellissier, 2001; Inderjit et al., 2010). As expected, recovery of many allelochemicals such as phenolic acids, saponins, isothiocyanates, and flavonoid glycosides spiked into soils is greater in sterile than non-sterile soils (Blum et al., 1994; Okumura et al., 1999; Weidenhamer and Romeo, 2004; Furubayashi et al., 2005;

Barto and Cipollini, 2009b; Chen et al., 2011). More surprising is the range of half-lives found for specific compounds in non-sterile soils. Measured half-lives of benzylglucosinolate range from 6 hours to 9 days (Gimsing et al., 2006, 2007); for 2-benzoxazolinone from 12 h to 30 days (Macías et al., 2004; Understrup et al., 2005); and for *p*-coumaric acid from 5 to 30 days (Blum et al., 1994; Pue et al., 1995). Factors that contribute to this variation include different degradation capabilities of microbial communities in different soil samples, abiotic soil characteristics such as pH (Gimsing et al., 2007; Johansen et al., 2007), the starting concentration of the allelochemical (Understrup et al., 2005; Kong et al., 2007; Gimsing et al., 2009), and the identities and concentrations of other organic compounds present in the soil (Blum et al., 1993; Pue et al., 1995; Blum, 1998; Macías et al., 2004). Half-lives are often modeled by using first-order kinetics, which implicitly states that the half-life is independent of starting concentration. This holds true for the portion of degradation driven by microbial enzymes, but the regular deviation of actual data from this model shows that microbial degradation sometimes operates under very limited conditions. Below a lower threshold, there may not be enough of the allelochemical to induce production of enzymes necessary for degradation, while toxicity of the compounds may limit microbial growth above an upper threshold (Macías et al., 2004). Further complicating matters is the fact that the lower threshold can increase if other organic compounds that are easier to digest are also present in the soil. The half-life of *p*-coumaric acid increased in the presence of glucose, thus demonstrating sequential carbon utilization where the energy rich glucose was degraded before *p*-coumaric acid (Pue et al., 1995). Phenylalanine and *p*-hydroxybenzoic acid also increased the half-life of *p*-coumaric acid, presumably through sequential carbon utilization as well (Pue et al., 1995). Allelochemical half-lives also can be increased by competition for degradative enzymes, and this was suggested as the mechanism behind the increased half-lives of two benzoxazinoids added to soil simultaneously (Macías et al., 2004). The importance of microbes in the degradation of allelochemicals is clear, and amazing progress is being made in understanding how interactions between allelochemicals and biotic and abiotic components of the soil matrix affect degradation.

Microbial Enhancement of Plant Tolerance to Allelochemicals Much of the reduction in allelopathic effects by microbes appears to be due to degradation of allelochemicals, but microbes also may increase plant tolerance of allelochemicals without actively degrading the offending compounds. Extracts of *Empetrum hermaphroditum* reduced nitrogen uptake in *Paxillus involutus* and *Pinus sylvestris* plants colonized by ectomycorrhizal fungi to one third that seen in controls, but nitrogen uptake in uncolonized plants was reduced to a tenth of the level seen in controls (Nilsson et al., 1993). Arbuscular mycorrhizal fungi also appear able to increase tolerance of plants to allelochemicals such as benzoxazinoids (Džafić et al., 2010) and glucosinolates and flavonoid glycosides (Barto et al., 2010a, b). Arbuscular mycorrhizal fungal spores are sensitive to allelochemicals produced by *Alliaria* (Callaway et al., 2008), as are AMF cultures *in vitro* (Koch et al., 2011), demonstrating that the fungus is not broadly tolerant of these allelochemicals. The North American native herbaceous plant, *Impatiens pallida*, also is sensitive to *Alliaria* allelochemicals, suffering reduced germination and growth when not associated with AMF. However, plants colonized by AMF grew similarly to controls, and had similar colonization levels when AMF were added to the system before allelochemicals (Barto et al., 2010a, b). Since the fungi were sensitive when not associated with the plant, it seems unlikely that they can degrade these allelochemicals on their own. It remains unclear whether the fungus increases plant tolerance, or whether both partners provide enzymes necessary for the degradation of these allelochemicals and that the complete pathway only exists in mycorrhizal plants. Symbiotic degradation of an allelochemical has been demonstrated by the degradation of the benzoxazinoid BOA by *Zea mays* only when colonized by a *Fusarium* endophyte (Knop et al., 2007). Interestingly, *Fusarium* growing in pure culture can initiate BOA breakdown (Yue et al., 1998), but when growing as an endophyte it will only produce enzymes for the later stages of BOA degradation, and it relies on its plant host to perform the first step (Knop et al., 2007). The increased tolerance of allelochemicals by plants colonized with AMF is less likely to be due to degradation of the allelochemicals by the fungus because the only carbon used by these fungi is provided by their plant hosts in the form of hexoses; the fungi are not thought to take up other carbon sources from the environment (Smith and Read, 2008). These fungi often improve plant nutrition and most likely increase tolerance of the plant to stresses such as exposure to allelochemicals by making it easier for stressed plants to recover.

Microbial Effects on Plant Allelochemical Production Microbes, such as foliar fungal pathogens (Fig. 1), also can reduce allelopathic effects by modifying the phytochemical profile of the allelopathic plant to reduce production of allelochemicals. *Ageratum conyzoides* infected with *Erysiphe cichoracearum* (powdery mildew) was less allelopathic on other herbaceous plants than uninfected plants, despite the fact that production of many volatiles was induced by infection (Kong et al., 2002). This result highlights the complexity of synergistic reactions among allelochemicals because many of the volatiles that were induced by *E.*

cichoracearum infection were allelopathic when applied alone (Kong et al., 1999). Although higher total amounts of volatile compounds were produced in infected plants, the mix of compounds was altered, and the concentration of precocene I declined significantly (Kong et al., 2002), suggesting that this compound may be especially important in the allelopathic effect of *A. conyzoides.* Infection by *Erysiphe cruciferarum*, a powdery mildew fungus that infects *Alliaria*, reduced the belowground competitive effects of *Alliaria* on a native herbaceous plant (Cipollini and Enright, 2009). Virulent strains of this fungus induce some generalized defense responses in leaves of *Alliaria* (Enright and Cipollini, 2011), but the profiles of putative phytochemicals involved in allelopathy belowground have not been compared between infected and uninfected individuals.

In summary, allelopathic effects can be greatly reduced by free-living soil bacteria and fungi, as well as by symbiotic and endophytic bacteria and fungi. These microbes act directly by degrading the allelopathic compound, and indirectly by increasing target plant tolerance of allelopathic effects and reducing production of allelochemicals by allelopathic plants. Going forward, future work should incorporate realistic microbial communities into experimental tests of allelopathy in order to better understand how microbes modify allelopathic effects. Important allelopathic effects in the field would appear to require that microbial protection mechanisms be overcome, and are perhaps restricted to areas with a limited history with the allelopathic plant.

Microbial Enhancement of Allelopathy

While the importance of abiotic soil factors with respect to the alteration and influence of allelochemicals cannot be stressed enough (Inderjit et al., 2010), microbial transformations and the general role of microbes with respect to allelochemicals clearly plays a major role in influencing allelopathic effects (Inderjit, 2005). In addition to detoxifying allelochemicals through degradation, however, soil microbial communities also have been shown to degrade toxic compounds into more toxic products (e.g., Gagliardo and Chilton, 1992) and to degrade relatively innocuous substances into toxic products. Allelopathic plants can modify plant-microbe interactions, resulting in increased allelopathic effects through increasing the sensitivity of target plants to pathogens and favoring growth of pathogenic or parasitic microbes. In addition, microbial communities can affect the allelopathic potential of a species or system in a more indirect way, such as the case of endophytic fungi that can stimulate allelochemical production by their host plants. Finally, networks of mycorrhizal fungi can distribute the toxic allelochemicals throughout plant communities, broadening their sphere of influence (Barto et al., 2011).

Increases in Allelochemical Toxicity through Microbial Degradation One of the more well-studied examples of the phytotoxicity of an allelochemical increasing through microbial modification comes from cereals in the Gramineae with consequences in agriculture, but also from plants in the Acanthaceae, Ranunculaceae, and Scrophulariaceae families. Many cereals produce the hydroxamic acids 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3(4*H*)-one (DIMBOA) and 2,4-dihydroxy-1,4-benzoxazin-3(4*H*)-one (DIBOA) (Niemeyer, 1988; Baumeler et al., 2000). The benzoxazolinones 6-methoxy-2-benzoxazolinone (MBOA) and 2-benzoxazolinone (BOA), interestingly enough, are the degradation products of DIMBOA and DIBOA, respectively (Fomsgaard et al., 2006). MBOA and BOA have been shown to be further transformed into 2-amino-7-methoxy-3*H*-phenoxazin-3-one (AMPO) and 2-amino-3*H*-phenoxazin-3-one (APO), respectively (Understrup et al., 2005). Gents et al. (2005) were able to demonstrate that the degradation of BOA to APO was concentration-dependent with low soil concentrations (400 μg kg^{-1}) yielding only one unidentified transformation product, while higher soil concentrations (400 mg kg^{-1}) yielded eight distinct transformation products, one of which was confirmed as APO in accordance with Gagliardo and Chilton (1992). APO has been shown to be more biologically active than BOA (Gagliardo and Chilton, 1992) including higher phytotoxicity and increased toxicity towards beneficial soil organisms and fungi (Gents et al., 2005). Gents et al. (2005) suggest that BOA is only toxic to microorganisms at higher concentrations and, therefore, microbes may convert BOA into APO and several other products thus ameliorating toxic conditions, thereby increasing phytotoxicity.

Allelopathic effects can be enhanced by microbial modification even when the parent compound is otherwise innocuous, with implications for plant invasions. Bains et al. (2009) found that exotic populations of *Phragmites australis* contained higher concentrations of the non-toxic gallotannin in their rhizospheres than native populations. These authors concluded that the invasiveness of the exotic *P. australis* can be partly explained by the increased levels of gallotannin that are degraded by native microbial and native plant communities into gallic acid, a much more biologically active and phytotoxic compound. Native plant and soil communities produce greater amounts of tannase, the enzyme responsible for hydrolyzing gallic acid from gallotannin, with greater activity than exotic *P. australis* plants. Therefore, it appears that *P. australis* exudes a relatively benign substance and relies on the surrounding microbial and plant communities to degrade it into a noxious compound, effectively freeing space (Bains et al., 2009) for further *P. australis* colonization. It is likely that gallic acid is further broken down to form even more phytotoxic products (Weidenhamer and Romeo, 2004), although it is unknown what role soil microbes play in this process. The findings reported by Bains et al. (2009) offer an interesting perspective on the Novel Weapons Hypothesis (Callaway and Ridenour, 2004; Callaway et al., 2008) in that a common compound that is produced at higher levels by an invasive plant genotype is "weaponized" by native soil microbial communities.

Microbial Infection and Realized Allelopathic Effects Indirect effects of allelopathic compounds include effects on microbial resistance of competitors. Voll et al. (2004) reported that extracts of *Brachiaria plantaginea* inhibited seed germination and root lengths of the two weed species, *Commelina benghalensis* and *Acanthospermum hispidum*. Interestingly, addition of *B. plantaginea* extracts to *C. benghalensis* seeds elevated levels of endophytic fungal infections and lowered germination rates. This study reveals the possible indirect allelopathic interaction that results when exposure to noxious chemicals enhances susceptibility of competing plants to microbial pathogens. Allelopathic plants in the Brassicaceae, especially *Brassica napus*, may also stimulate soil populations of plant pathogens like *Pythium* (Hoagland et al., 2008), which are expected to reduce the growth of surrounding plants. Mycorrhizal associations also can be modified by allelopathic plants, as shown for *Molinia caerulea*, which appears to limit growth of an efficient ectomycorrhizal symbiont in *Picea abies* roots while stimulating growth of a more parasitic symbiont instead (Timbal et al., 1990). It remains to be seen how the spread of invasive plants is enhanced by the allelopathic modification of microbial interactions with native plants.

Endophytic fungi also have been shown to enhance the allelopathic potential of plants, possibly by producing allelochemicals for their plant hosts or by stimulating allelochemical production (Fig. 1). Pyrrolizidine alkaloids were produced by *Festuca* only when infected by an endophyte (Malinowski et al., 1999), and are likely produced by the endophytes and provided to the plants. A similar mechanism may be behind the results of Vásquez-de-Aldana et al. (2011), who showed that root exudates of red fescue (*Festuca rubra*) had a greater inhibitory effect on the germination and seedling growth of four target species when infected with a fungal endophyte, *Epichloë festucae*. Endophyte infection can be beneficial to the host plant with respect to many factors including increased resistance to drought, salt stress, nematodes, mammalian and insect herbivores, and bacterial and fungal pathogens (Kimmons et al., 1990; Li et al., 2009; Ownley et al., 2010; Sabzalian and Mirlohi, 2010; Miranda et al., 2011; Rocha et al., 2011). Rudgers and Orr (2009), however, were able to show that soils conditioned by non-native tall fescue (*Lolium arundinaceum*) that was infected by the fungal endophyte, *Neotyphodium coenophialum*, were able to reduce the biomass of *Elaeagnus umbellata*, *Fraxinus pennsylvanica*, and *Platanus occidentalis*. This reduction in biomass was apparent only in live soil treatments suggesting that above and below-ground microbial interactions are important in this system.

Microbial Enhancement of Allelochemical Distribution The existence of mycorrhizal networks may enhance allelopathic effects of plants, especially if the mycorrhizae themselves are insensitive to the allelochemicals. Despite early evidence that the invasive forb *Centaurea stoebe* was more allelopathic towards *Festuca idahoensis* when mycorrhizal than when un-colonized (Marler et al., 1999), it does not appear that *C. stoebe* was parasitizing *F. idahoensis* by drawing carbon through the mycorrhizal network (Zabinski et al., 2002). In this specific case, Zabinski and coworkers attributed the invasiveness of *C. stoebe* to its ability to outcompete native grasses with respect to exploiting mycorrhizal relationships more effectively, and, therefore, being better able to capture resources from soil. More broadly, Barto et al. (2011), suggest that common mycorrhizal networks facilitate the transfer of allelochemicals from donor to target plants. In two separate experiments, these authors showed enhanced accumulation of an herbicide, imazamox (representing a hydrophilic allelopathic compound), in tissues of target plants (*Zea mays*) connected to the dosing site by a common mycorrhizal network. In order to eliminate concerns about the application of compounds in unrealistic amounts as well as simulating a natural donor-target plant system, these authors also used the allelopathic *Tagetes tenuifolia* which exudes hydrophobic phytotoxic thiophenes from its roots. Again, allelochemical concentrations in soils were significantly greater and target plant biomass lower with common mycorrhizal networks present. Transfer of plant available P was not a factor in these experiments (Barto et al., 2011), in contrast to the results found by Zabinski et al. (2002). Barto et al. (2011) was the first study to clearly demonstrate the role that soil communities can have with respect to the amplification and expansion of plant-plant allelopathic effects and in light of these findings, future allelopathy studies must incorporate these principles and control for the effects of common mycorrhizal networks.

Though not the specific subject matter of this review, it is worth mentioning studies such as Meier and Bowman (2008) that showed that certain allelochemical fractions of *Acomastylis rossii* increased soil respiration, reduced *Deschampsia caespitosa* growth, and reduced *D. caespitosa* N concentrations. The explanation these authors offered was that the availability of labile sources of C stimulated microbial activity, which, therefore, reduced the availability of N to *D. caespitosa*. This is an indirect way in which microbial processing of soil C sources can indirectly mimic allelopathic effects, but does not necessarily require "toxic" allelochemicals to be present. For a review of the ecosystem function and biogeochemistry in plant invasions, see Weidenhamer and Callaway (2010).

Mechanisms by which microbes can enhance allelopathic effects may be at least as important in community dynamics (i.e., plant invasions), as the mechanisms described above where microbes reduce allelopathic effects. The importance of these alternative mechanisms likely varies in different systems, and specific research is needed to understand how these opposing mechanisms interact in the field.

Conclusions and Outlook

Allelopathy has been an increasingly popular topic of study for the past 50 years, with almost 4000 articles indexed by ISI. Microbes have been included in these studies only in the past 30 years, and fewer than 300 publications have been indexed by ISI in that time. Within this short period, much progress has been made towards understanding how microbes act as targets and mediators of allelopathy in plants. Allelopathic inhibition of beneficial microbes such as mycorrhizal fungi, rhizobia, and plant growth promoting rhizobacteria also indirectly limits growth of the plants hosting those microbes. Microbes whose growth is not directly affected by allelochemicals still often mediate effects of those compounds on other plants, both reducing and increasing allelopathic effects. Reductions in allelopathic effects occur through several mechanisms that include microbial degradation of allelochemicals, an increasing tolerance of colonized plants to the stress of exposure to allelochemicals, and the modification of the phytochemical profiles of allelopathic plants that reduces allelochemical production. Increases in allelopathic effects also can be driven by microbial degradation of natural products when the products of degradation are more toxic than the parent compounds, through modifications of plant microbe interactions, and by microbial induction of allelochemical production by plants. Furthermore, bioactive zones of allelochemicals are increased in soils with intact arbuscular mycorrhizal fungal networks, which seem to serve as ‘highways’ for allelochemical movement directly from donor to target plants.

It is becoming increasingly apparent that the degree and direction of microbial mediation of allelopathy will not remain constant through time. Much of the research on allelopathy in natural systems is focused on invasive plants, which are usually non-native species. The Novel Weapons Hypothesis posits that non-native invasive plants are more able to exert allelopathic effects on naïve plants in the invaded range because the invader and the native plants do not share a co-evolutionary history (Callaway and Ridenour, 2004). This same logic can be applied to naïve microbes in invaded ranges because they are less likely to have evolved mechanisms to degrade allelochemicals or affect allelochemical production than microbes in the native range of invaders. As microbes become more adapted to both the novel weapons and the non-native plants producing them, allelopathic effects are likely to decrease in some cases, but increase in others. Beneficial microbes that are sensitive to allelochemicals will likely develop resistance over time, and the indirect inhibitory effects on the plants associating with them will, therefore, decline. Likewise, microbes that act to reduce allelopathic effects by degrading allelochemicals will likely become more efficient as they adapt to the novel allelochemicals (Blum and Shafer, 1988; Walker and Welch, 1991). Conversely, microbial enhancement of allelopathic effects through increased toxicity of microbial degradation products is predicted to increase as the time since invasion increases. Microbial reduction of allelopathic effects through increased tolerance of the target plant is unlikely to change through time if the microbes are not sensitive to the allelochemicals, but could increase as native microbes develop resistance to any inhibitory effects of the allelochemicals.

Effects of time since invasion on microbial modification of the phytochemical profile of non-native allelopathic plants are more difficult to predict. Production of allelochemicals by *Alliaria* appears to decline with time since invasion (Lankau et al., 2009), but it is unclear what role microbes play in this reduction. Arbuscular mycorrhizal fungal communities in areas invaded by *Alliaria* are beginning to shift away from those found in uninvaded areas (Burke, 2008; Lankau, 2011b; Barto et al., 2012). Bacterial communities are not always affected by *A. petiolata* invasion (Burke and Chan, 2010), but where microbial communities are affected, resistance to *Alliaria* allelochemicals has begun to develop in only 50 years since the invader was introduced (Lankau, 2011b). Another allelopathic invasive plant, *C. stoebe*, also modifies microbial communities, including effects on rhizosphere bacteria and arbuscular mycorrhizal fungi associating with neighboring plants (Batten et al., 2006; Mummey and Rillig, 2006; Broz et al., 2009). This modification of the microbial community is not short term (Kulmatiski and Beard, 2011) and likely takes years to develop, as has been shown for *Alliaria*.

Microbes can serve as targets and mediators of allelopathic effects in plants, with both strong positive and negative effects being possible. More effort should be made in the future to include microbes in allelopathy research to improve ecological realism. Ecological realism is attained partly by using non-sterile soils in assays of allelopathic effects, using natural inputs of allelochemicals, followed by examination of microbial community changes. In addition, soil sterilization followed by substitutions of different portions of the microbial community could be used to identify important classes or specific species of microbes that modify allelopathic effects. These studies also could be enhanced by using representatives of plant species that are differentially dependent on specific microbes, and done in different soil types to examine abiotic influences. Selection experiments could also be performed to evaluate evolutionary responses of microbes to allelochemicals, and more work on adaptation of specific microbes or microbial populations to allelopathic plants is needed in the field. Further exploration of microbial communities in the native range of invasive plants could aid predictions of microbial adaptation, community shifts, and the “longevity” of allelopathic effects in invasive ranges. Knowing the extent of departure of the microbial communities from the native range of an invasive plant from that found in a “preinvaded” range, coupled with the extent of departure of allelochemistry in the invasive plant from relatives in the preinvaded range (e.g., Barto et al., 2010b), may aid predictions of the potential allelopathic effects of invasive species. All of this work should be coupled with improvements in sampling and analytical techniques for allelochemicals and microbes in the environment.

In an applied sense, research is needed to explore the potential of reintroducing microbes to areas where allelopathic plants have taken hold as a part of restoration plans. In turn, potentially negative effects of microbial reintroduction (or controlled inoculations in agricultural systems) on allelochemical modification should also be explored. Much additional research is needed on how allelochemical degradation is affected by the presence of other plant-derived compounds, since this can both increase and decrease allelopathic effects. Understanding how seemingly disparate mechanisms interact in the field will become increasingly important. In situations where allelopathic effects are seen in natural environments, microbial protection mechanisms are apparently being overcome by other, often undefined, mechanisms. A deeper understanding of the factors that control the balance between reducing and enhancing effects could allow development of management schemes targeted for specific areas that could reduce the impact of allelopathic plants without actively removing them. Likely candidates for these controlling factors include microbial species identities and abundances, neighboring plant species identities and abundances, along with abiotic factors such as soil organic matter content, nutrient levels, and water holding capacity. Additionally, the contribution of allelopathic effects to the phenomenon of plant-microbe feedback is still unclear, even for plants with known effects on soil biota. Finally, the movement of allelochemicals through common mycorrhizal networks provides a mechanism for greatly enhanced delivery of bioactive doses of allelochemicals to target plants, but it is still unclear how important this mechanism is in the field. Interest in microbial mediation of allelopathic effects is only expected to increase in the future, and the field is ripe with questions.

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REVIEW ARTICLE

Ant Interactions with Soil Organisms and Associated Semiochemicals

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Abstract This review focuses on the semiochemical interactions between ants and their soil environment. Ants occupy virtually every ecological niche and have evolved mechanisms to not just cope with, but also manipulate soil organisms. The metapleural gland, specific to ants was thought to be the major source of semiochemical antimicrobial compounds targeting general or specific deleterious microbes. The extremely diverse variety of semiochemicals and their sources with antimicrobial activity or potential activity is highlighted. The leaf-cutting ants and fire ant provide the most researched species, in part because they cause significant economic damage. The leaf-cutting ant is particularly interesting because researchers have uncovered unexpected interactions between leaf-cutting ant fungal farm, parasitic fungi, bacteria, yeasts, and ant defensive semiochemicals. These complex relationships highlight the multidimensional aspects of ants and the soil environment in which they live.

Keywords Formicidae · Microbes · Fungi · Bacteria · Yeast · Parasite · Semiochemical · Defense

Introduction

Social insects are characterized by three factors: 1. Individuals of a species cooperate in taking care of immature forms; 2. Reproductive division of labor occurs where non-reproductive individuals work for fecund individuals; and 3. There is an overlap of generations such that offspring assist parents. Insect species exhibiting all of these traits often are referred to as eusocial. Only some highly evolved wasps and bees, but all ants and termites are eusocial. Termites are restricted to habitats with cellulose resources, but ants, the subject of this review, have evolved to take advantage of a wide variety of resources and inhabit virtually every terrestrial/arboreal ecological niche–worldwide. There are over 14,000 extant described ant species (Hymenoptera: Formicidae) in 21 extant and 5 extinct sub-families and 298 genera (see Fig. 1). There are an estimated 8,000 species yet to be described (Bolton 2012). The number of scientific citations from the FORMIS (51,000 references) database (Wojcik and Porter 2012) associated with extant and extinct subfamilies is highly correlated with the number of species in each subfamily (Fig. 1). However, certain genera (and species) within the three major subfamilies dominate, e.g., about 75 % of the Myrmicinae citations are due to 4 of 141 genera: *Solenopsis*, *Atta*, *Acromyrmex*, and *Monomorium*; about 75 % of subfamily Formicinae citations are due to 2 of 49 genera: *Formica* and *Camponotus*; and in subfamily Dolichoderinae, about 64 % of the citations are due to 2 of 6 genera: *Linepithema* and *Tapinoma*.

Ants do not fly except when winged female and male sexuals have mating flights. Thus, they are primarily terrestrial although some species occupy arboreal habitats. Terrestrial ant colonies come in all sizes from tens of workers to millions; from a single queen to multiple queens; form a variety of nests types from leaf litter to deep intricately excavated earthen nests that are works of art to the human eye (Tschinkel 2004, 2010). In solitary (non-social) insects, males and females have evolved a wide variety of mechanisms (including pheromones) to find each other, mate, and produce a new generation of reproductive males and females. For ants, the colony is the reproductive entity,

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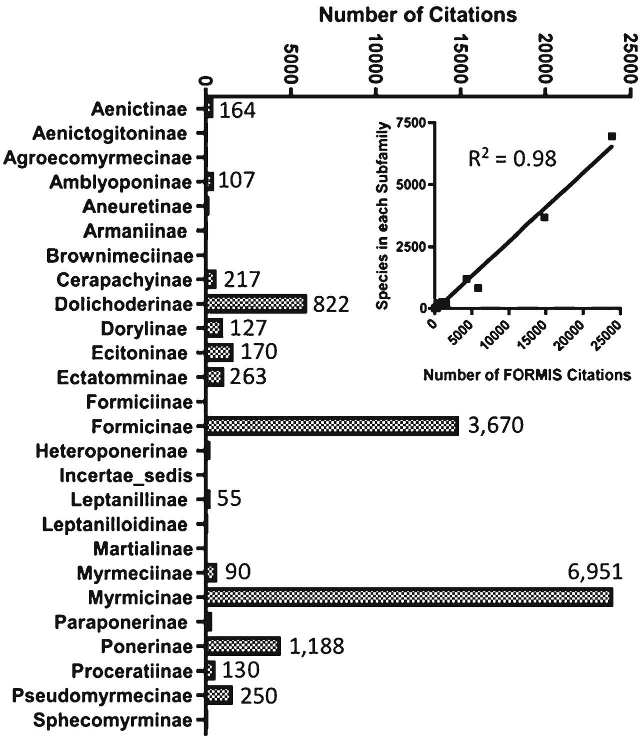

Fig. 1 Number of citations in Formis (Wojcik and Porter 2012) associated with each of the 21 extant and 5 extinct Formicidae subfamilies. The number of species and subspecies in each subfamily is shown above or near the subfamily bar (Bolton 2012). Linear regression of species in each subfamily vs. the number of citations was highly significant (inserted graph)

and is comprised of the mated queen(s) that may live for a decade or more and a non-reproductive worker force that brings in resources for the queen and developing brood, maintains nest structure, and defends the colony (queen) from outside intrusions. A colony can lose 90 % of the worker force and the colony will recover. Once mature, an ant colony usually undergoes annual ergonomic (worker production) and reproductive (sexual production) stages until the queen dies.

Soil is home to a wide variety and huge numbers of organisms that for a particular ecological niche attempts to maintain homeostasis. In this milieu of soil organisms, ants represent an important group of macro-organisms that dramatically affect the soil in which they live. For example, they are second in animal turbation only to earthworms: 5,000 $gm^{-2}\ y^{-1}$ vs. 15,000 $gm^{-2}\ y^{-1}$ for earthworms, but ants likely are more important because their geographic distribution is much wider than that of worms (Paton et al. 1995), e.g., in the deserts of Australia, soil turnover rates are reported to be 420 $kg\ ha^{-1}\ y^{-1}$ (Briese 1982). On the other end of the spectrum, in Argentina, *Camponotus punctulatus* worker ants moved 2,100 $kg\ ha^{-1}\ y^{-1}$ of soil in improved pastures (Folgarait 1998). In addition to soil turbation, the soil in the vicinity of ant mounds usually is enriched with nutrients due to the concentration of resources needed to maintain their large populations (Dostál et al. 2005; Wagner and Jones 2006; Wagner and Fleur Nicklen 2010).

The above highlights the dynamic and intimate nature of the interaction between ants and soil, thus it is not surprising that ants have a high probability of contacting a myriad of soil microorganisms, some of which are mutualistic, symbiotic, or entomopathogenic. The latter, often highly coevolved interactions, represent a classic "arms race" where ants have evolved defenses similar to those for non-social insects, such as avoidance behavior and use of the integument as a protective shield from pathogens. Once parasite penetration occurs, an immune response may be elicited that can take many forms, e.g., biochemical, innate, and molecular (Brennan and Anderson 2004; Schmidt et al. 2008). Specifically for ants, *Formica rufa* and *Myrmecia gulosa* respond to bacterial challenge by producing antimicrobial "defensin" peptides, 40 amino acids long (Taguchi et al. 1998) and 2×16 amino acids long (Mackintosh et al. 1998), respectively. In *Lasius neglectus*, transfer of *Metarhizium anisopliae* spores from infected to naïve workers through grooming leads to up-regulation of "defensin" and prophenoloxidase (PPO) in the previously naïve workers, resulting in sub-lethal infections. *Solenopsis invicta* responds to premeditated fungal infection by up-regulating the transferrin gene, an immune response (Valles and Pereira 2005; Schlüns and Crozier 2009).

Social insects rely on a wide variety of semiochemicals for maintenance of social structure, territory, defense, and food procurement. Illustrative of their potential complexity are the 75 recently described exocrine glands (Billen 2009a), including 20 from ant legs (Billen 2009b). See Fig. 2 for a few of these exocrine glands. Some of these exocrine glands are involved in chemical defense and may directly influence organisms in their underground habitat. While many of these exocrine glands are probably involved in the lubrication of moving parts, there is ample documentation for the synthesis of a wide variety of structural types (Morgan 2008), as well as functions (Vander Meer and Alonso 1998). This review will focus on the semiochemicals

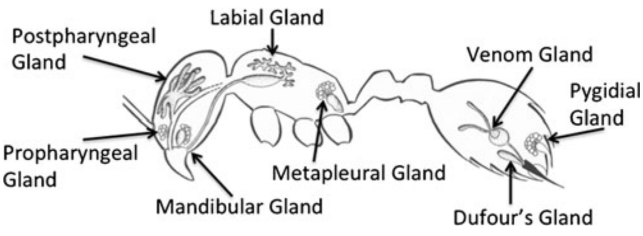

Fig. 2 Common exocrine glands of ants are shown in this schematic profile drawing of an ant. Mandibular, Dufour's, pygidial, and venom glands are known to produce pheromones. The metapleural gland produces antibiotics. Drawing by Johan Billen, University of Leuven, Belgium

and other compounds that may influence ant and soil microorganism interactions.

The Metapleural Gland

The metapleural gland (MG) is unique to ants (Hölldobler and Engel-Siegel 1985) and is located in the metathorax (Fig. 2). Secretory cells open to a holding chamber that leads to the surface through an always-open orifice, thus gland products can flow freely to the outside cuticle (Schoeters and Billen 1993). The location of the gland is conducive for worker ants to use their legs to apply the exocrine products to themselves, nestmates, and/or substrates within the nest. This gland produces compounds that have antibiotic activity (Maschwitz et al. 1970; Maschwitz 1974) that could function to protect terrestrial ant colonies from soil pathogens.

A comparative study (Hölldobler and Engel-Siegel 1985) has shown that not all ant species have functional MGs, e.g., arboreal ants, slave-making ants, and males of many species. All of these apparent anomalies have been rationalized by hypothesized lower pathogen exposure levels (arboreal species), functional usurped colony workers (slave raiders), or alternative uses where males have functional glands (Hölldobler and Engel-Siegel 1985). Other functions for the MG have been proposed, e.g., nestmate recognition (Brown 1968) and territorial marking (Jaffe and Puche 1984). Certainly with over 14,000 described ant species, multiple functions are not unexpected, especially since functional parsimony is well documented in social insects (Blum 1996; Trhlin and Rajchard 2011; Matsuura 2012, this issue). These areas are interesting, however, I restrict this review to ant relationships with soil microorganisms and ant derived antimicrobial activity starting with MGs and adding other chemistry that relates to ant/microorganism interactions. See Yek and Mueller (2011) for a comprehensive review of the metapleural gland in ants.

Metapleural gland secretions from all ant species investigated (12) have anti-bacterial activity (Yek and Mueller 2011), but in limited screening, *Pseudomonas* and *Bacillus* bacteria species were resistant to the secretions (Iizuka et al. 1979; Mackintosh et al. 1995). Metapleural glands from three Attine leaf-cutting ants (2 × *Atta* and 1 × *Acromyrmex*) and one bulldog ant (*Myrmecia*) species have been evaluated against 18 fungi species. Five fungi were resistant to MG secretions, primarily from *Atta sextens* (4 of 5). The five resistant fungi were entomopathogenic (2) or phytopathogenic (3) (Yek and Mueller 2011). It is clear that MG secretions have antibiotic activity, but the activity is not uniform against potential pathogens (Yek and Mueller 2011). The chemistry associated with MG products generally has acidic characteristics, expressed in the form of carboxylic acid or phenol moieties. Examples of these along with the ant species they are associated with are shown (Table 1, Fig. 3).

The remainder of this review is ordered based on ant subfamilies.

Subfamily Myrmicinae

The Attines

All Attines are fungus (Basidiomycota: Agaricales) growing species. The lower and higher Attines differ in that the lower Attine fungus gardens (families Pterulaceae/Lepiotaceae) can survive independently from the ants, whereas the higher Attines have evolved a sophisticated obligate mutualism with the family Lepiotaceae fungi that supply the ants with a required food resource. The ants culture the fungus by providing necessary physical conditions and plant substrates needed for its growth (Villesen et al. 2004; Schultz and Brady 2008). In this review, higher Attine species are designated Attine, and the lower Attines are indicated as necessary. The Attine species are of special interest for this review. The group is composed of four major genera (Bolton 2012): *Trachymyrmex* (47 spp. and 1 subspecies), *Sericomyrmex* (19 spp. and 3 subspecies), *Atta* (15 spp.), and *Acromyrmex* (30 spp.). *Atta* and *Acromyrmex* species are major leaf harvesters in the neotropics and cause significant economic problems (Cherrett 1986; Fowler et al. 1986), which have resulted in much basic and applied research. The obligate mutualism has been a driving force in MG investigations, because not only do the Attines need a way to combat microorganisms that negatively affect their workers and other life stages, but their obligate fungi also are susceptible to parasitism, usurpation, and/or mortality from competing organisms. Therefore, it is possible that antibiotic compounds produced in the MG could serve to protect both colony members and their co-evolved highly specific fungal garden. The two functions have different requirements: general antibiotic activity works for protection of colony members; however, protection of the specific fungal garden requires a targeted rather than a generalist antibiotic.

Forty-three compounds have been identified from Attine MGs, primarily from *Acromyrmex octospinosus* (Ortius-Lechner et al. 2000, 2003) and to a lesser extent from *Ac. subterraneus*, *Atta sextens*, and *A. cephalotes* (Schildknecht 1976; Beattie et al. 1984; do Nascimento et al. 1996). Compounds A, B, and C (Table 1, Fig. 3) are often most abundant. Component A, 3-hydroxyl decanoic acid (myrmicacin) has antimicrobial activity (Iizuka et al. 1979), but the compound is not specific to Attines (see Table 1), and no activity against antagonists of the obligate fungal garden has been demonstrated. Indoleacetic acid (compound B, Table 1, Fig. 3) is interesting in that it is a well-known plant growth

Table 1 Identified components from the metapleural glands of ant species are indicated by an X. Column 2, Structural Type, corresponds to the letter under each structure in Fig. 3. For *C. difformis*: x = Attygalle et al. 1989; X = Jones et al. 2005. Compounds <1 % not included

Metapleural gland components	Structure type	*Atta sextens*	*Acromyrmex octospinosus*	*Ac. subterraneus*	*Myrmica rubra*	*Messor barbarus*	*Crematogaster defformis*	*Crematogaster inflata*	*Solenopsis invicta*
3-hydroxyldecanoic acid (myrmicacin)	A	X	X	X	X	X			
3-hydroxyloctanoic acid	A-1	X							
3-hydroxylhexanoic acid	A-2	X							
Indoleacetic acid	B	X	X	X	X				
Phenylacetic acid	C	X			X	X			
4-oxo-octanoic acid	D		X						
4-oxo-decanoic acid	D-1		X						
γ-octalactone	E		X						
3,4-diH-8-hydroxyisocoumarin	F								
Mellien	G						X		
6-propylsalicylic acid	H						xX		
6-pentylsalicylic acid	H-1						X		
6-heptylsalicylic acid	H-2						X		
6-pentyl resorcylic acid	I						X	X	
6-heptyl resorcylic acid	I-1						X	X	
6-nonyl resorcylic acid	I-2						X		
5-propyl resorcinol	J						xX		
5-pentyl resocinol	J-1						xX	X	
5-heptyl resorcinol	J-2							X	
5-nonyl resorcinol	J-3								
3-propyl phenol	K						xX		
3-pentyl phenol	K-1						xX		
3-heptyl phenol	K-2						x		
Carboxylic acid	L		X						X

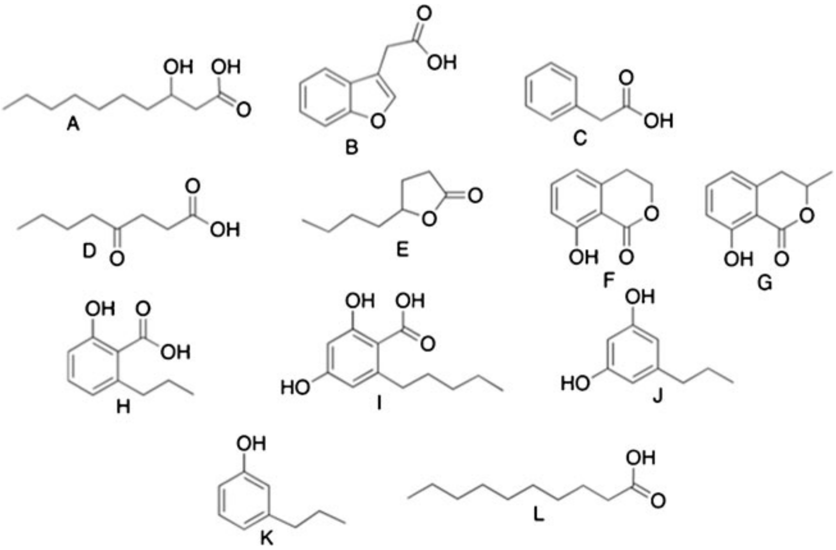

Fig. 3 Representative structures for compounds isolated from the metapleural gland of ants. Compound letters match the Structure Type column in Table 1. See Table 1 for the structural name and the species from which the compounds were isolated

hormone (Wrightman 1962), and is a major MG component in *Ac. octospinosus*, but a minor component in *A. sextans* and *A. cephalotes*, except for soldiers (18 %) of the latter species (do Nascimento et al. 1996). Phenyl acetic acid (Component C) also is a plant auxin (Wightman and Lighty 1982) and a major component in the MG of *A. sextans* and *A. cephalotes*, but it is absent from *Acromyrmex* species (do Nascimento et al. 1996). These three compounds individually and in combination have been evaluated for their effects on the growth of the fungal garden of *A. sextans*. Both growth enhancement and inhibition were observed depending on concentrations, suggesting that workers may be able to stimulate garden growth or increase usage of MG chemistry to cope with a negative invader, even at the expense of fungal garden growth (Schildknecht et al. 1973). After contacting the MG, *Atta* and *Acromyrmex* spp. groom themselves, each other, and their fungal gardens, distributing the MG products. In addition, when *Atta* workers are challenged with foreign fungal conidia, they respond by increasing their grooming rate, and thus MG product distribution (Fernández-Marín et al. 2006). Interestingly, ants in general have evolved an excellent particulate filtration mechanism for ingested liquid food or from the grooming process (Eisner and Happ 1962; Glancey et al. 1981). Particles are compressed by the mouthparts into the infrabuccal pocket where a pellet is eventually formed and ejected. *Atta* workers with their MGs sealed produce fewer infrabuccal pellets than normal workers. Conidia in pellets from workers with sealed MGs germinated with greater frequency than those from normal workers. This demonstrated the effect of MG products, and that workers actively respond to microorganism challenge with increased use of MG secretions (Fernández-Marín et al. 2006). Another fungal challenge experiment has shown that small *Ac. echinatior* workers have more developed MGs than large workers and are better capable of resisting size equivalent spore doses (Poulsen et al. 2006).

Attine MG secretions likely play a role in protecting workers from microbial attack, in maintaining fewer microorganisms in the nest soil, and even in sustaining the integrity of the fungal garden; however, they must act in concert with other mechanisms and probably other exocrine gland products. For example, Attine mandibular glands (Fig. 2) contain citral, 4-methyl-3-heptanol,, 2-heptanone, 3-octanone, 4-methyl-2-heptanone, β-citronellol, and geraniol (Blum et al. 1968). All of these compounds except 4-methyl-2-heptanone and β-citronellol exhibit anti-fungal activity as great as the MG components: phenylacetic acid, indoleacetic acid, hexanoic and octanoic acids (Mendonça et al. 2009). Recently, 3-octanol, 3-octanone, 3-heptanone, 4-methyl-3-heptanone, perillene, and α,α-acariolide were isolated from one or more of ten Attine *Trachymyrmex* and one *Sericomyrmex* head extracts (Adams et al. 2012), likely mandibular gland products. Abdominal extracts yielded *E*, *E*-α- and *Z*-β-farnesene along with alkanes (Adams et al. 2012). The trail pheromone of some *Atta* and *Acromyrmex* species has been determined to be from the poison gland, and a common active component was methyl 4-methylpyrrole-2-carboxylate (Tumlinson et al. 1972; Evershed and Morgan 1983). In addition, some *Atta* species also produced 3-ethyl-2,5-dimethylpyrazine (see Fig. 4) in their poison glands (Evershed and Morgan 1983), thus illustrating the diversity of exocrine gland compounds available to influence Attine surroundings. In an Atta species, 29 unique worker tasks have been identified including: caring for hyphae, licking substrate, implanting hyphae, transporting hyphae, and degrading vegetation, all activities that could involve both mandibular gland, MG, and or other glandular products (Wilson 1980). It is clear that we are far from understanding the synergies and interactions of multiple glandular products

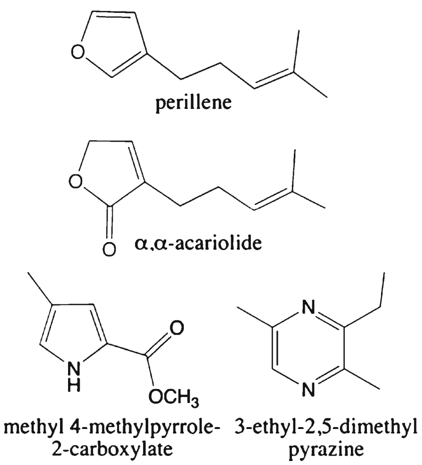

Fig. 4 Perillene and α,α-acariolide were isolated from Attine head (Mandibular glands?) extracts (Adams et al. 2012). The pyrrole and pyrazine are trail pheromone components isolated from Attine poison glands (Evershed and Morgan 1983)

in the complicated world of the Attines, and other ant species.

The last publication that dealt with new chemistry and components of Attine MGs was over 10 years ago (Ortius-Lechner et al. 2000). Lack of a clear association between MG components and Attine fungal gardens, along with the fascinating discovery that the Attines use antibiotic producing bacteria to control parasites of their fungal garden (Currie et al. 1999), shifted and multiplied research efforts to the latter subject.

The fungal gardens of Attine species are attacked by highly co-evolved micro-fungi parasites (*Escovopsis* spp.) that if unchecked can quickly destroy the fungal garden. The parasitic fungi is specifically attracted to chemicals (unknown structures) released by its host fungal garden, as evidenced by preferential growth of the *Escovopsis* parasite toward their fungal host rather than to non-host fungi (Gerardo et al. 2006). Fungi are known to produce volatiles that attract insects (Pierce et al. 1991; Schiestl et al. 2006) and/or have antimicrobial activity, e.g., penicillin. In addition, the *Escovopsis* strain isolated from host A will move quicker toward host A cultivar than toward other host cultivars, perpetuating *Escovopsis* strain and host fungal garden fidelity and the mutualism between the two fungi (Gerardo et al. 2006).

A bacterium has been isolated from fungus garden material that inhibits germination of the conidia of a *Escovopsis* sp. parasite and several entomopathogenic fungi, but not the mutualistic fungal garden of the ant (Santos et al. 2004). This is the magic bullet that gets around the apparent non-specific antibiotic activity of MG products. Filamentous Actinomycete bacteria from *Pseudonocardia* (Currie et al. 1999), *Streptomyces* (Haeder et al. 2009), and *Burkholderia* (Santos et al. 2004) genera have been isolated from fungal gardens. Remarkably, Attine workers have evolved specialized cuticle structures (crypts) and associated glands that hold these special bacteria–essential in the Attine defense against *Escovopsis* spp. parasites (Currie et al. 2006; Haeder et al. 2009). An antifungal macrocyclic compound has been visualized on the cuticle of workers by using a MALDI imaging technique, thus demonstrating that these symbiotic bacteria can protect workers from microbes, while the workers actively or passively distribute the bacteria within their nest and fungal garden (Schoenian et al. 2011). The special cuticular structures support a co-evolution developmental process; however, the actinomycete symbionts may be the result of frequent environmental acquisition rather than co-evolution (Mueller et al. 2008; Barke et al. 2011).

Importantly, several antifungal compounds have been isolated and identified from Attine symbiotic bacteria, e.g., Candicidin-D (*Streptomyces* spp.), Antimycin (*Streptomyces* spp.). Structures of these compounds are shown in Fig. 5. Valinomycins, antimycins, and actinomycins have been identified from *Streptomyces* isolates from three *Acromyrmex* spp. via LC-MS (Schoenian et al. 2011). Each of these compound groups shows varying activities against fungi, including the *Escovopsis* parasite of the Attine obligate mutualist fungus, as well as bacterial *Streptomyces* and *Pseudonocardia* symbionts. Antifungal compound combinations exhibit synergistic effects against the fungal garden parasite, *Escovopsis* (Schoenian et al. 2011). Similarly, candicidin and antimycin fungicides are made by a *Streptomyces* bacterial symbiont of *Ac. octospinosis*. Mutants of this *Streptomyces* that were unable to produce candicidins or antimycins still maintained activity against the *Escovopsis* parasite, indicating that additional antimicrobial compounds are being produced (Seipke et al. 2011).

To add further complexity to Attine symbiotic relationships (at this point–ants: bacteria: fungal garden: parasitic fungus) was the discovery of a black yeast (Ascomycota; *Phialophora*) growing on the worker cuticle of a lower Attine species (*Apterostigma*), which was localized on the cuticle with the symbiotic bacteria (Little and Currie 2007). The black yeast could not be isolated (cultured) from other Attine genera; however, the yeast was detected from Attines by using molecular techniques. A related molecular phylogeny study showed that the black yeasts from the Attines formed a derived monophyletic group, thus supporting the hypothesis that the yeasts are symbionts of the Attines (Little and Currie 2007). This represents a fifth symbiotic relationship. These same authors further demonstrated that the black yeast received nutrients from the mutualistic bacterial biomass on the ants (Little and Currie 2008). Yeast manipulation experiments have demonstrated that yeast infected colonies have reduced capacity to produce antifungal agents (via the bacteria),

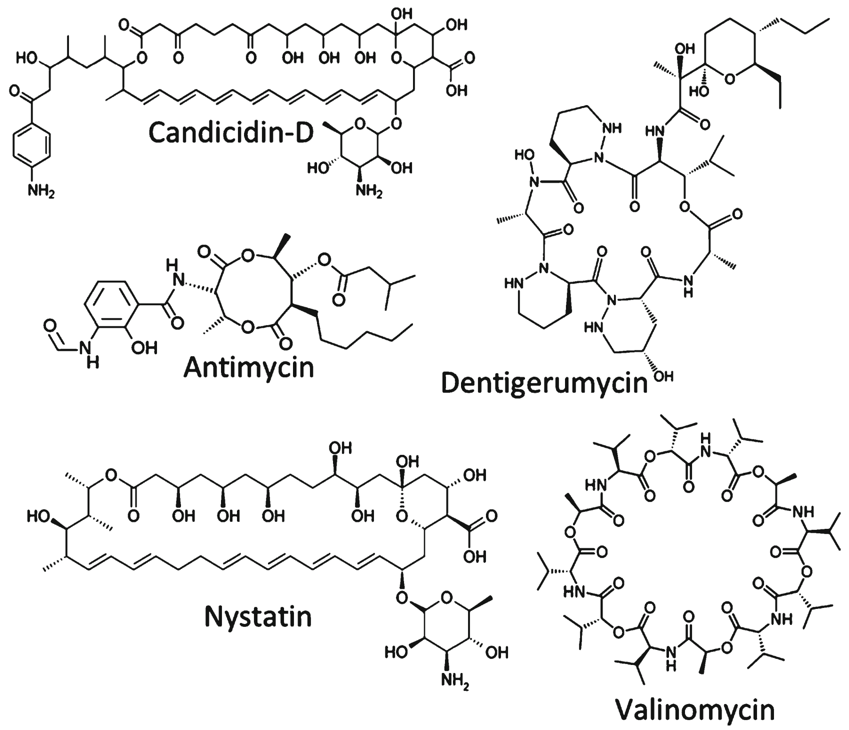

Fig. 5 Five anti-fungal agents isolated from leaf-cutting ant Actinomycete symbiotic bacteria. spp., Dentigerumycin (*Pseudonocardia* spp.), Valinomycin (*Streptomyces* spp.) and a Nystatin-like compound (Haeder et al. 2009; Oh et al. 2009; Barke et al. 2010; Schoenian et al. 2011)

with a consequent reduction in their ability to defend against the parasitic fungi (*Escovopus*), which in turn lowers fungal garden/colony health (Little and Currie 2008).

There are many more chapters to go in this intriguing and complicated story of Attine survival in the soil environment. The real challenge is understanding the dynamics of what has become a powerfully linked community of symbionts, mutualists, and parasites to create a holistic picture that includes the interactions of Attine worker behaviors, exocrine gland products, suites of symbiotic bacteria, obligate host fungal gardens, specific fungal garden parasites, and black yeasts. While experimentally it is expeditious to take a reductionist approach and isolate parts of complex systems, e.g., ants and fungal cultivar, we all too often stop there and neglect to attempt to put the parts together in a holistic approach. Attine research forces this issue, as exemplified by the informative web diagram constructed by Little and Currie (2008) that helps visualize the complex sets of interactions associated with Attine ants, bacteria, yeast, fungal cultivar, and cultivar parasite.

Solenopsis–Fire Ants

Solenopsis currently has 183 described species (Bolton 2012). Most of these are "thief ants" that have small monomorphic workers living in small colonies, generally near nests of other ant species where they manage to steal that colony's food and brood (Thompson 1989). The *Solenopsis saevissima* species group is comprised of 20 new world species that have very different characteristics from the thief ants (Pitts et al. 2005). Members of this group generally are called fire ants, due to the burning sensation associated with their stings. *Solenopsis richteri* and *S. invicta* are the most notorious of the fire ants, because they were accidentally introduced into the United States in the 1910s and 1930s, respectively, into Mobile, Alabama, where *S. invicta* quickly outcompeted *S. richteri* and adapted to the lack of natural enemies and non-competitive native ant species (Wilson 1958; Lofgren 1986). The ant spread through natural mating flight activities, but most aggressively through human movement of infested nursery stock throughout the southern United States (Lofgren 1986). Imported fire ants now infest more than 130 million ha in 13 southern tier states and Puerto Rico and are spreading northward (APHIS 2011). In the last decade, *S. invicta* has changed from an invasive pest ant in the United States to a global problem, with infestations occurring in Australia (Henshaw et al. 2005), Taiwan (Chen et al. 2006), mainland China (Zeng et al. 2005; Zhang et al. 2007), Mexico (Sánchez-Peña et al. 2005), and many Caribbean Island countries (Davis et al. 2001).

Fire ants are a central foraging species that has a nest tumulus made from excavated below ground soil. Nests may extend 2–3 m in depth. Mature colonies can have up to 250,000 workers and a density of 120 mounds/ha (monogyne social form). Underground foraging tunnels radiate from the central nest with exit holes to the surface (Markin et al. 1975). However, resource gathering occurs both on the surface and underground (Tennant and Porter 1991).

Fire ants use a variety of exocrine gland (Fig. 2) products to maintain social structure and defend territory, e.g., Dufour's gland regulates foraging (Vander Meer 1986b), queen poison gland induces retinue activity (Vander Meer et al. 1980), mandibular gland products alarm workers (Vander Meer et al. 2010), and worker poison gland products are used in defense and food procurement (Obin and Vander Meer 1985). The subterranean habitat of fire ant colonies puts them at risk from soil microbes and nematodes.

Metapleural Gland Based on previous examples, fire ant metapleural glands would be expected to have antimicrobial activity. The metapleural gland chemistry of *Solenopsis invicta* and *S. geminata* has been reported as a series of four fatty acids: palmitic, linoleic, oleic, and stearic acids, along with minor amounts of hydrocarbons, which are attributed to substances (cuticular hydrocarbons) accumulating in the MG reservoir from the cuticle (Cabrera et al. 2004). Although neither MG extracts nor their individual components have been evaluated for antibiotic activity at physiological concentrations, some fatty acids do negatively impact bacteria (Kabara et al. 1972). When minimum inhibitory concentrations from Kabara et al. (1972) are compared with the amount of each of the four carboxylic acids found by Cabrera et al. (2004), it is clear that one (*S. geminata*) or two of the acids (*S. invicta*) are at a concentration high enough to inhibit bacterial growth (Table 2), especially since the MG reservoir volume is likely much less than 1 µl. The hypertrophied MG reservoir volume for *Crematogaster inflata* is about 0.1 µl (data in Billen et al. 2011).

Poison Gland Chemistry Some 54 years ago, the chemical and antibiotic properties of fire ant venom were reported (Blum et al. 1958), and a venom alkaloid was later unambiguously identified as *trans*-2-methyl-6-n-undecylpiperidine (MacConnell et al. 1970). Since these first reports the chemistry of the major alkaloid components (Fig. 6) have been well defined as series of *cis*- and/or *trans*-2-methyl-6-alkyl or alkenyl piperidines (MacConnell et al. 1971; Brand et al. 1972) that vary with each fire ant species, such that they can be used as chemotaxonomic characters (Brand 1978; Vander Meer et al. 1985; Vander Meer 1986a). The USA native fire ant, *S. xyloni*, in addition to the *cis* and *trans* piperidines, also has a detectable amount of 2-methyl-6-n-undecyl-$\Delta^{1,2}$-piperideine (Fig. 6), which was suggested previously as a likely precursor to the piperidines or as an intermediate in the inter-conversion of the *cis* and *trans* ring isomers (Brand et al. 1972). The absolute configuration of the alkaloids was determined as shown in Fig. 6 (Leclercq et al. 1994). Biosynthetic studies have demonstrated that the piperidine alkaloids are biosynthesized via a polyacetate route and that, indeed, 2-methyl-6-n-undecyl-$\Delta^{1,6}$-piperideine and/or 2-methyl-6-n-undecyl-$\Delta^{1,2}$-piperideine are intermediates to the piperidines (Leclercq et al. 1996). With current separation and detection systems, the piperideines can be observed in extracts of *Solenopsis* poison sacs, but are present in ≤1 % of the total alkaloid (personal observation). Nevertheless, publications on piperideines that have appeared lately have provided support for the Leclercq et al. (1996) biosynthetic scheme (Chen et al. 2009; Chen and Fadamiro 2009a, b).

Table 2 Carboxylic acid Minimum Inhibitory Concentrations (MIC) for 8 bacteria species and 2 strains (Kabara et al. 1972) compared with the amount of four carboxylic acids found in *S. geminata* and *S. invicta* MGs (Cabrera et al. 2004). NI = Not Inhibitory

Carboxylic acid	MIC (ng/µl)	From *S. geminata* (ng/gland)	From *S. invicta* (ng/gland)
Palmitic	122 (6/10)[a]	47	206
Stearic	NI (0/10)	11	11
Oleic	500 (1/10)	29	207
Linoleic	12.3 (7/10)	69	184

[a] Number of inhibited bacteria/out of a total of 8 bacteria and 2 strains (Kabara et al. 1972)

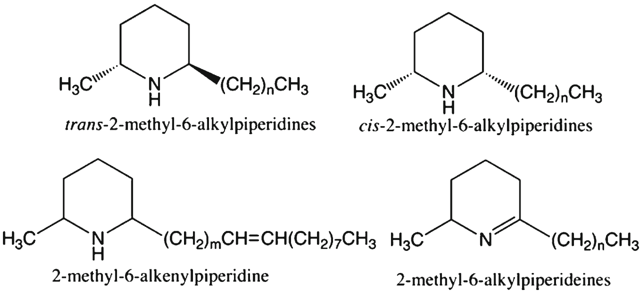

Fig. 6 Structures of common piperidine alkaloids produced by fire ants (*N*=10, 12, 14, 16; *m*=3,5,7). The *trans*- and *cis*- isomers have the configurations shown (Leclercq et al. 1994). The piperideines are generally minor compounds and are considered to be biosynthetic precursors to the piperidines (Leclercq et al. 1996)

Fire ant piperidine alkaloids have wide ranging effects on humans, from platelet and neutrophil activation (Javors et al. 1993) to lytic release of histamine from mast cells causing the characteristic sterile pustule formation at the site of the sting (Lind 1982). The venom of stinging Hymenoptera had been shown previously to contain proteins, thus fire ant venom appeared to be an outlier, even though many cases of allergic reactions and anaphylactic shock were being reported from fire ant stings (Lockey 1974; Rhoades et al. 1977). This conundrum was solved when small amounts of proteins were isolated (≈0.1 % of venom weight) and characterized (Baer et al. 1979) as similar to those found in other hymenopteran venoms, namely with phospholipase and hyaluronidase activity. Research on the fire ant allergens continues (Padavattan et al. 2008), driven by the fact that 1 % of the human population is susceptible to developing hypersensitivity to fire ant stings and about 33 % of the population in the infested areas are stung each year, resulting in significant medical demands (Partridge et al. 2008).

Interestingly, the fire ant has evolved a variety of mechanisms for release of venom alkaloids to the environment, generally called “gaster flagging” (Obin and Vander Meer 1985). In the context of defense, the ants raise their gaster 90° to the substrate and vibrate it while slowly emitting venom from the sting. This is a well-known mechanism for generation of an aerosol (Mason et al. 1963). Small amounts of venom reach the intruder who may be repelled and a physical confrontation avoided. This sequence has been observed when another ant species has surrounded a food source and a single foraging fire ant uses the aerosol mechanism to repel the other ants from the food (Banks and Williams 1989). A similar mechanism has been reported for *Monomorium minimum* (Adams and Traniello 1981). If the aerosol does not work, fire ant workers can accurately throw 0.03 to 0.13 mm diameter droplets at an approaching enemy and, of course, as a last resort they will attempt to bite and sting an intruder (Obin and Vander Meer 1985). Most relevant for this review is the use of gaster flagging within the context of the nest and readily observed in laboratory colonies near brood, where about 1 ng of alkaloid per piece of brood has been detected (Obin and Vander Meer 1985). It is assumed that the ants are also dispersing venom alkaloids on nest soil as a prophylactic or in response to pathogen challenge, although this has not been directly demonstrated.

The antimicrobial activity of fire ant piperidine alkaloids, first described by Blum et al. (1958) was later shown to have greater inhibitory activity against gram-positive than against gram-negative bacteria (Jouvenaz et al. 1972). Fungicide, termiticide, and seed germination inhibition have been added to the growing repertoire of piperidine alkaloid activities (Escoubas and Blum 1990). Germination of the entomopathogenic fungi, *Beauveria bassiana*, *Metarhizium anisopliae*, and *Paecilomyces fumosoroseus* are inhibited at physiologically relevant alkaloid concentrations (Storey et al. 1991). In the field, biodiversity of fungi in fire ant nest soil is less (50 %) than the surrounding soil, although fungal abundance is greater than in non-nest soil (Zettler et al. 2002). Therefore, the fire ant influences the fungal make-up of the nest soil directly, via venom alkaloids, MG carboxylic acids, and/or other undiscovered mechanisms. Another option related to what was already seen with the Attines is that the remaining fungi or bacteria in the fire ant nest soil may inhibit growth of other fungal species (Domsch et al. 1980). There is support for this: when nest soil and sterile soil with fire ants were inoculated with conidial suspensions of entomopathogenic fungus, *Beauveria bassiana*, the LC_{50} conidial concentration for nest soil was 2×10^7 greater than that for sterile soil, thus supporting the concept that fire ant nest soil organisms are producing fungicidal metabolites (Shields et al. 1981; Pereira et al. 1993). Direct evidence is needed.

Recall that MG chemistry and antimicrobial activity are linked to acidic moieties. In contrast, the fire ant piperidine alkaloids are basic and have wide ranging physiological activities that include antimicrobial activity. The fire ant MG produces acidic antibiotics, while the poison gland produces basic antibiotics. The effects of a combination of acidic and basic antibiotics with soil microbes have yet to be determined.

Solenopsis–*Thief Ants*

Thief ant species are a large world-wide, taxonomically difficult group previously classified in the subgenus, *Diplorhopturum* (Pacheco 2001). Workers are diminutive and live almost exclusively underground where their resources come from larvae stolen from other ant species. These ants, not unlike their larger fire ant relatives, have evolved an excellent recruitment system, as well as defensive chemistry that incapacitates defending workers of the species raided, allowing them to escape with brood (Blum et al. 1980). Across the thief ant species thus far investigated, the venom chemistry shows a diverse array of alkaloid structural types and a variety of isomers within each type. Figure 7 shows an example of each structural type: pyrrolidine (Jones et al. 1979), pyrrolizidine (Jones et al. 1980), indolizidine (Gorman et al. 1998), quinolizidine (Jones et al. 1999), decahydroquinoline (Jones et al. 1999), and pyrroline (Jones et al. 1982). These compounds have been of interest because of their interesting role in food procurement, potent defense against other ant species, possible chemotaxonomic

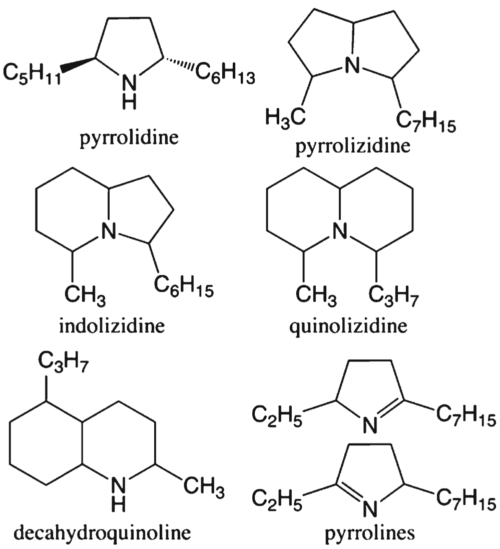

Fig. 7 *Solenopsis* spp. thief ant poison glands produce a wide variety of alkaloids as illustrated by these representative compounds: 2-hexyl-5-pentylpyrrolidine, 3-heptyl-5-methylpyrrolizidine, 3-hexyl-5-methylindolizidine, 4-methyl-6-propylquinolizidine, 2-methyl-5-propyldecahydroquinoline, and 2-heptyl-5-ethylpyrroline (see text for references)

utilization, and the high probability that new alkaloid natural products would be discovered. Insecticidal activity has been-reported for pyrrolidines, pyrrolines, pyrrolizidines, and indolizidines (Bacos et al. 1988; Escoubas and Blum 1990). The former two also inhibit seed germination (Escoubas and Blum 1990). Thief ants also have to cope with microorganisms in their soil habitat; however, their metapleural gland chemistry is unknown. The impressive alkaloid diversity, their demonstrated physiological activities, and evidence that plant-derived derivatives of the alkaloid classes shown in Fig. 7 also have antimicrobial activity, suggests a nest hygiene role for thief ant venom components.

Monomorium

The *Monomorium* genus has over 400 species and subspecies. The Pharaoh's ant, *M. pharaonis*, is the most well-known species in this genus because it is an invasive pest in many parts of the world and has been implicated in transmission of pathogenic bacteria (Hughes et al. 1989). Species in this genus have characteristics similar to thief ants, e.g., they use venom to repel other ant species away from resources (Hölldobler 1973). Investigation of *Monomorium* venom components have shown them to be similar to those alkaloid classes shown in Fig. 7 for *Solenopsis* thief ants, e.g., pyrrolidines, indolizidines, pyrrolines (Jones et al. 1982), and unsaturated pyrrolizidines from the related species *Chelaner antarcticus* (now *Monomorium*) (Jones et al. 1986). Novel dialkylpiperidenes (Jones et al. 1990) and monosubstituted pyrrolines (Jones et al. 2003) can be seen in Fig. 8. As with *Solenopsis* thief ants, *Monomorium* spp. can use these alkaloids for prey procurement and defense (Hölldobler 1973). The defensive utility can be against other ant species or soil microbes.

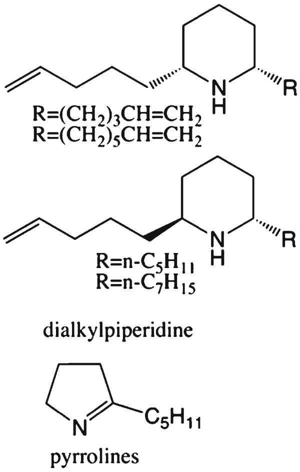

Fig. 8 *Monomorium* species produce a similar suite of compounds to those shown for *Solenopsis* thief ants in Fig. 7. In addition, they produce the specific examples of dialkyl piperidines and mono substituted pyrroline shown in this Figure (Jones et al. 1990, 2003)

Crematogaster

The genus *Crematogaster* has 771 species and subspecies. *Crematogaster difformis* and *C. inflata* are arboreal ants living in myrmecophytic epiphytes in the crowns of canopy trees (Tanaka et al. 2009) and tree cavities (Billen et al. 2011), respectively. Since they do not have the purported pressure that soil inhabiting ant species have to produce antimicrobial defenses, their MGs were expected to be non-functional and atrophied (Hölldobler and Engel-Siegel 1985). Instead, their MGs are highly developed; in fact, *C. inflata* has the largest known MGs (measured by secretory cell counts, Billen et al. 2011). Metapleural gland chemistry has been reported for *C. difformis* in two reports, one using samples collected from Malaysia (Attygalle et al. 1989) and the other from Brunei (Jones et al. 2005). The MG chemistry of each is so unique (combinations of 6-alkylsalicylic and 6-alkylresorcylic acids, 5-alkylresorcinols and 3-alkylphenols; see Table 1, Fig. 3) that Jones et al. (2005) suggested that they are likely different species. Comparison with a third species, *C. inflata* (Jones et al. 2005), shows a third blend of components (Table 1, Fig. 3). Metapleural gland chemistry may provide a useful chemotaxonomic character for classifying members of this large and difficult genus. Interestingly, the *C. inflata* MG secretion has been described as viscous (Buschinger and Maschwitz 1984; Ito et al. 2004) and did not inhibit growth of *E. coli*, whereas the secretion of *C. difformis* is fluid and inhibited growth of *E. coli* (Maschwitz 1974). Different functions have been ascribed to each MG secretion, predator and pathogen defense, for *C. inflata* and *C. difformis*, respectively (Buschinger and Maschwitz 1984; Ito et al. 2004). The gland chemistry of both species, though different from Attines, has the typical carboxylic and phenol acidic moieties (Table 1, Fig. 3) associated with the antibiotic role of MGs (see Yek and Mueller 2011). The classes of compounds produced, 6-alkylsalicylic acids (Gellerman et al. 1969); 5-alkylresorcinols (Ayer et al. 1983; Jin and Zjawiony 2006); and related alkylphenols (Fontenelle et al. 2011) have been shown to have antibiotic activity. These data demonstrate that MG products that have antibiotic activity also can have negative effects on predator arthropods and *vice versa*. It is well-known that epiphytes and tree cavities harbor a wide assortment of fungi and bacteria (Gönczöl and Révay 2003; Hashidoko 2005; Yuan et al. 2009), so it follows that *Crematogaster* species or other ants that nest in tree cavities or myrmecophytic epiphytes or any related habitat have a need for protection from pathogens and predators. Likewise, other MG antibiotic compounds may also function as deterrents to predators–functional parsimony is a common feature in social insects (see Matsuura 2012, this issue). Pheromone function is usually named after the first discovered function, e.g., alarm pheromone, but this sometimes acts like blinders to a horse, retarding the discovery of additional functions.

Pogonomyrmex

The *Pogonomyrmex* genus is composed of 77 species. Ant species that come in contact with the human population–such as the fire ant and those species that change human behavior after being stung–like the "pogos" usually initiate characterization in the context of their physiological effects on people. Harvester ants (*Pogonomyrmex* spp.) have one of the most painful and long lasting stings in the ant world (3 of 4 with 4 = "traumatically painful") (Starr 1985). The components are phospholipases, lipase, hyaluronidase, phosphatase, and several esterases, histamine, and kinin-like peptides (Schmidt and Blum 1978a). A pharmacological and toxicological study of the venom of *P. badius* found it to be the most toxic known insect venom to mice (Schmidt and Blum 1978b). Unfortunately, no antimicrobial evaluations have been conducted. *Pogonomyrmex* live in large colonies underground where defense against microbes is expected to be important.

Aphaenogaster and Messor

The *Aphaenogaster* and *Messor* genera are composed of 215 and 161 species, respectively. The poison glands of several *Aphaenogaster* species contain anabaseine and/or anabasine (Fig. 9), alkaloids also found in tobacco and related to nicotine, e.g., *A. fulva* and *A. tennesseensis* (Wheeler et al. 1981); *A. rudis* (Attygalle et al. 1998); *A. subterranea* and *A. miamiana* (Leclercq et al. 2001). Recently, the poison sac of *A. senilis*, but not *A. iberica* was found to contain anabasine and anabaseine, as well as other unidentified alkaloids (Lenoir et al. 2011). Anabaseine hydrochloride was shown to have strong antibacterial and fungicidal activity (Abdulina et al. 2002), suggesting that *Aphaenogaster* poison gland products could function in nest defense against predators, parasites, competitor ant species, and control microbes in their nest environment. Members of the *Messor* genus also produce antibiotic anabasine (Fig. 9) and several pyrazines as minor components of their poison gland (Cruz López et al. 2006).

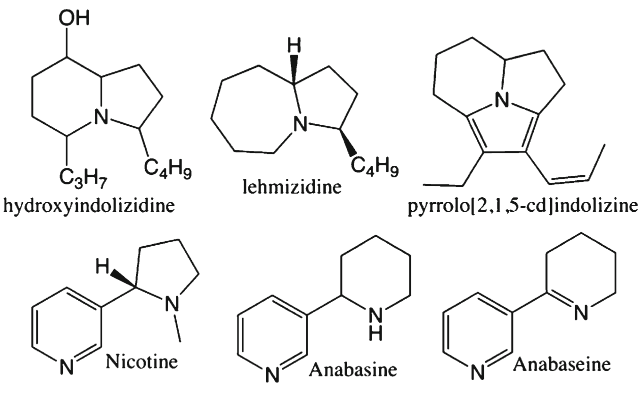

Fig. 9 The genus, *Myrmicaria*, produces alkaloids similar to *Solenopsis* thief ants and *Monomorium* species (Fig. 7), as well as the top three compounds shown here (Jones et al. 2007; Schröder et al. 1996). The poison glands of *Aphaenogaster* and *Messor* genera produce anabaseine and/or anabasine, analogs of nicotine

Myrmica

The *Myrmica* genus contains 171 species and 2 subspecies. Plant auxins, indoleacetic acid (Schildknecht and Koob 1970) and phenyl acetic acid were detected from MG extracts of *Myrmica rubra* (initially named *M. laevinodis*, but since changed to *M. rubra*), as well as 3-hydroxydecanoic acid (myrmicacin)(Schildknecht 1976). In the same report, *Messor barbarus* was shown to have phenylacetic acid and myrmicacin, but not indoleacetic acid (see Table 1, Fig. 3). All three compounds inhibit fungal (*Botrytis cinerea* and *Altenarea tennis*) sporulation, with myrmicacin especially effective (Schildknecht 1976).

Myrmicaria

The *Myrmicaria* genus is comprised of 67 species. *Myrmicaria melanogaster* is phylogenetically close to *Monomorium* and *Solenopsis* thief ants, and their venom alkaloids show similarities and differences (Jones et al. 2007). They produce pyrrolidines, indolizidines (Fig. 7), and a novel hydroxylated indolizidine, a mono-substituted lehmizidine (Jones et al. 2007), and a tricyclic alkaloid, pyrrolo[2,1,5-cd]indolizines (Fig. 9), as well as dimeric and trimeric versions of the tricyclic alkaloid (Schröder et al. 1996). Antimicrobial activity has not been directly investigated.

Myrmecia

The *Myrmecia* genus is composed of 89 species. These Australian bulldog ants are noted for their aggressive behavior and potent sting. *Myrmecia pilosula* ranks high on the human pain scale (2–3) (Starr 1985). Research has identified allergens (Wiese et al. 2007). A separate study targeting antimicrobial activity, identified a cytotoxic peptide (Zelezetsky et al. 2005), pilosulin 1, from the venom of *M. pilosula*, that had strong antifungal and antibacterial activity. Although obvious, the general point is that chemistry function is driven by bioassay. We have already seen that ants are capable of regulating the release of their venom into the environment, so it would not be surprising if low-level dispersal of protein dominated venoms also play a role in nest hygiene. Research is needed.

Subfamily Formicinae

Camponotus and Calomyrmex

The *Camponotus* and *Calomyrmex* genera are composed of over 1,500 and 14 diverse species/subspecies, respectively. The *Camponotus* genus generally does not have a functional metapleural gland, rationalized by the fact that carpenter ants do not live in the soil, but prefer moist decaying wood; therefore, microbial pressure is not as great as on the soil inhabiting ants. They may not have the same problems, but rotting wood is an excellent habitat for a variety of microbes, fungi, bacteria, and yeasts (Blanchette and Shaw 1978; Zhang et al. 2008) including Actinobacteria that produce Attine fungicides (Barke et al. 2010) and basidiomycetes fungi that are related to the Attine fungal garden. Chemical analysis of the contents of mandibular glands from *Camponotus quadrisectus* and *C. irritibilis* (collected from Brunei) led to the identification of three new structures, *E*-2-ethyl-2-hexen-1-ol, 4-hydroxymellein, and *E*-2-ethyl-2-hexenal, as well as mellein (Fig. 10) as major components for one or the other species (Voegtle et al. 2008). There also are minor amounts of m-cresol and 6-methyl salicylic acid. Mandibular glands are usually associated with alarm and defense, but Voegtle et al. (2008) noted that the major mandibular gland products have antibiotic activity (Kanoh and El-Gindi 2004; Lyr and Banasiak 1983) expanding possible roles for the mandibular gland products of *Camponotus* species and subfamily Formicinae in general. In support of the above, an undescribed *Calomyrmex* species produces antibiotic substances from their mandibular glands. Pyrazines were likely responsible for the antibiotic activity (Brough 1983).

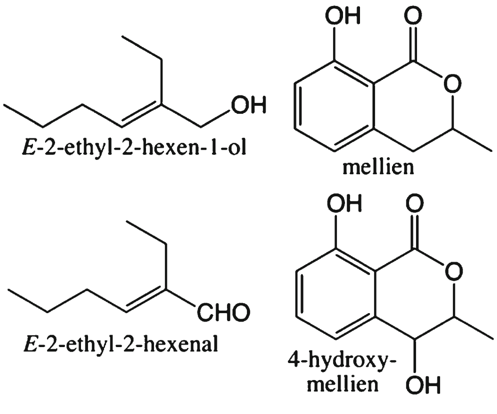

Fig. 10 *Camponotus* is a large genus in the subfamily Formicinae. They do not have a functional MG; however, the mandibular gland products shown here have antibiotic activity (Kanoh and El-Gindi 2004; Voegtle et al. 2008)

Another possibility: Formic acid is the major component in the venoms of all species of ants in the Formicinae subfamily. Formic acid is cytotoxic, present in concentrations up to 60 %, and the venom also contains small peptides and amino acids (Blum 1984). These ants do not sting, but they have powerful mandibles that can pierce the skin, during which they curl their abdomen toward the bite and spray formic acid. Carpenter ants can control the release rate and direction of release of their venom. Formic acid has antibiotic activity and could contribute to control of soil microbes.

Subfamily Dolichoderinae

Linepithema

The *Linepithema* genus contains 20 species. *Linepithema humile* (previously known as *Iridomyrmex humilis*), the Argentine ant, is a pest species in many parts of the world. The pygidial gland (Fig. 2) is used for defense in this subfamily. The pygidial glands of *L. humile* contain iridomyrmecin and dolichodial (Cavill et al. 1976); the former was demonstrated to be a strong insecticide and have antibiotic activity (Pavan 1949). The antibiotic activity of iridoids from plants have been shown to have a broad activity spectrum (Bakuridze et al. 1987). In addition, the Argentine ant produces a number of pyrazines in the Dufour's glands (see Fig. 11 for structures). Pyrazines have been shown to have antibiotic activity (Premkumar and Govindarajan 2005).

Subfamily Ponerinae

Pachycondyla

The genus *Pachycondyla* contains 252 species and 62 subspecies. One of these species, *Pachycondyla goeldii*, is typical of the subfamily in that it is an aggressive predatory ant that relies on its venom for subduing its prey. The venom was found to contain 15 peptides named ponericins, all of which show antibacterial activity (Orivel et al. 2001). Three structural types have been identified–ponericins G, W, and L. Broad physiological activities were found, including hemolytic, insecticidal, antibacterial (gram positive and negative), and yeast inhibition. The authors suggest that besides prey procurement, these ants are cleaning the prey of microbes prior to being brought into the colony, which presents a second possible function for these broadly active peptide venom components.

Summary and Needed Research

While the four ant subfamilies cited here (out of 21) represent the greatest number of species (about 90 % of the 14,300

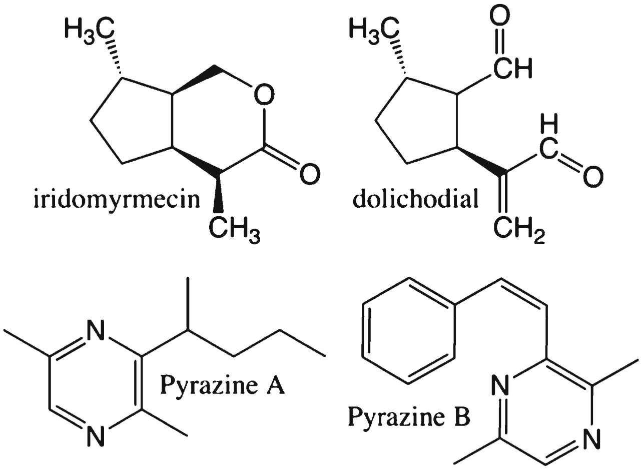

Fig. 11 The Argentine ant is the most studied species in the *Linepithema* genus. The pygidial gland produces compounds shown here. Iridomyrmecin has been shown to have antibiotic activity (Pavan 1949) and the other compounds by analogy are likely to also have antibiotic activity (Bakuridze et al. 1987; Premkumar and Govindarajan 2005)

described ant species) and citations (see Fig. 1), less than 1 % of those species are mentioned in this review. There is a lot of relevant literature that could not be included, and there obviously is a lot more work to be done. Consider this review an appetizer, not a meal. My objective was to explore the complexities of soil organism–ant interactions, which certainly are highlighted by the Attine system, and to indicate where additional research is needed. The latter can be overwhelming.

As I have pointed out in this review, research related to chemical ecology is needed in several areas. For example: a) What are the products and functions of the Attine glands associated with the cuticular crypts that harbor the symbiotic bacteria? b) Are Attine worker products from the mandibular gland and/or other exocrine glands involved in the preparation of cut leaves for the fungal gardens? If so, what is their function? c) What is the chemistry involved in attraction of the *Escovopsis* parasite fungus to its host Attine symbiotic fungus? d) What are the antibiotic effects of combinations of exocrine gland products? Fire ants produce both acidic (MG) and basic (poison gland) antibiotic compounds. How do they interact in the soil/microbial environment? This same question likely applies to most ants in the Myrmicinae subfamily. e) We know little about MG chemistry, despite the fact that it has been highlighted in this review. f) Exocrine gland products can have multiple functions—the same products may elicit defense against microbes and predators/intruders, but this needs to be demonstrated. g) Ants are capable of regulating the release of their venom into the environment, thus low-level dispersal of alkaloid or protein-dominated venoms could play a role in nest hygiene. This has not been demonstrated. h) We have seen that arboreal and decaying wood-inhabiting ant species do have a need for antimicrobials that could be met through their mandibular or venom gland products, but this needs confirmation. i) In many examples, the chemistry reveals interesting natural product structures, but the function is assumed, not demonstrated, and other functions, such as antibiotic activity, rarely have been investigated.

The beauty of working with ants is that with over 14,000 described species evolving in virtually every ecological niche in the world, it is impossible to generalize and surprises are not the exception. Other areas of research not addressed in the review that also need attention are itemized below:

A) *Cross talk between microorganisms* via *semiochemicals.* This is a developing area of research with a variety of potential outcomes (Shank and Kolter 2009) that is even more exciting when linked to the complexities of the Attine system.

B) *Entomopathogenic nematodes.* These were not addressed here, but some, of course, are specialists on ants (Poinar et al. 2007; Rasman et al. 2012, this issue) and have been considered for biological control of some pest ant species. Besides the ant–nematode interactions, nematophagous fungi are also part of the picture (Soto-Barrientos et al. 2011).

C) *Parasites that manipulate the behavior of their ant hosts, thus optimizing their development.* Zombie ants—A *Camponotus* sp. infected with *Ophiocordyceps* fungi exhibits predictable altered zombie-like behavior that benefits the parasitic fungus (Hughes et al. 2011). *Ophiocordyceps* infections of *Atta* and *Acromyrmex* species have been reported (Hughes et al. 2009).

D) *Ant Viruses.* The first ant viruses were identified using a metagenomic approach, specifically for the fire ant, *Solenopsis invicta*. Three RNA viruses have been isolated and characterized (Valles 2012). The first two, SINV- 1 and SINV-2 are found in all developmental stages, but only chronic asymptomatic infections have been observed, except that SINV-1 sometimes becomes pathogenic if colonies are stressed, e.g., dug up and brought to the lab. A third virus, SINV-3 has proven to be virulent in colonies brought back to the lab and is readily transmitted to healthy colonies through air and mechanical transfer. The virulence has not been observed in the field, but laboratory colonies are short-lived. The main difference, of course, is the soil and the possible negative interactions between soil microorganisms and SINV-3 that may keep the virus in check under field conditions. Another virus has been discovered recently from the crazy ant, *Nylanderia pubens* (Valles et al. 2012) indicating that ant viruses may be common. Virus–soil interactions may be important factors in ant colony health (Yeager and O'Brien 1979).

It should be clear that ants are chemical factories that produce a huge diversity of structural types that have evolved with all the pressures of the unique, dynamic soil, wood, or arboreal environment encountered by each species. These pressures have led to multiple exocrine gland semiochemical sources that can have multiple contextual functions. It is no wonder that ants are poor pollinators–they are walking pharmacies that just casual contact with can reduce the viability of flower pollen (Beattie et al. 1984). There is no predicting what new chemistry you will find when working with a new ant species, which is why ants have been such a rich source for new natural products. Imagine a fire ant worker covered with hard cuticular armor, oozing with antibiotic carboxylic acids from its metapleural gland, sending off an antibiotic/anti-predator aerosol of poison gland piperidine alkaloids through its sting into the nest environment, releasing pyrazine alkaloid alarm pheromones from its mandibular glands at the slightest disturbance, depositing minute amounts of sesquiterpene and homosesquiterpenes from the Dufour's gland to mass recruit workers to food or initiate colony emigration. These worker ant activities are based on independent pieces of research. We tend, by necessity, to be reductionist in our research, but the leaf-cutting ant story rings out loud and clear that the real world is a dynamic multidimensional system in a delicate equilibrium. Understanding the dynamics is one of our greatest challenges.

> Korean kimchi was recently defined as a "mix of cabbage with spices and salt brine: several strains of naturally occurring *Lactobacillus* will start pumping out acids that lower the pH, killing off pathogens and imparting a mouth tingling tang." (Wired, 060, May 2012).
>
> Leaf-cutting ants had the concept first!

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REVIEW ARTICLE

Multifunctional Queen Pheromone and Maintenance of Reproductive Harmony in Termite Colonies

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Abstract Pheromones are likely involved in all social activities of social insects including foraging, sexual behavior, defense, nestmate recognition, and caste regulation. Regulation of the number of fertile queens requires communication between reproductive and non-reproductive individuals. Queen-produced pheromones have long been believed to be the main factor inhibiting the differentiation of new reproductive individuals. However, since the discovery more than 50 years ago of the queen honeybee substance that inhibits the queen-rearing behavior of workers, little progress has been made in the chemical identification of inhibitory queen pheromones in other social insects. The recent identification of a termite queen pheromone and subsequent studies have elucidated the multifaceted roles of volatile pheromones, including functions such as a fertility signal, worker attractant, queen–queen communication signal, and antimicrobial agent. The proximate origin and evolutionary parsimony of the termite queen pheromone also are discussed.

Keywords Termite queen pheromone · Semiochemical · Pheromone parsimony · Caste differentiation · Primer pheromone

Introduction

Termite (the order Isoptera) consists of over 2,600 species worldwide (Kambhampati and Eggleton 2000). They are grouped according to their feeding behavior in the categories: subterranean, soil-feeding, drywood, dampwood, and grass eaters. Subterranean termites build an intricate network of tunnels in soil, connecting multiple nest sites and wood. Most pest species of subterranean termites in the Northern Hemisphere belong to the genera *Reticulitermes* and *Coptotermes*. Due to their cryptic habitat in soil, subterranean termites are difficult not only to control but also to study. Especially, their population ecology and reproductive biology largely remain to be understood.

The sophisticated colony organization of eusocial insects is maintained primarily through the utilization of pheromones. Queen-specific pheromones have several functions within the colony, serving as releaser pheromones (behavioral activators) and primer pheromones (physiological primers). Releaser pheromones, which change the probability of performing a certain behavior upon perception, can elicit queen-tending behavior in workers, ensuring that the queens are groomed and fed and that their eggs are cared for. They also can elicit aggressive policing behaviors in workers against individuals producing a competing reproductive signal within a colony. Primer pheromones have the potential to suppress nestmate fertility (Hoover et al., 2003; Le Conte and Hefetz, 2008). Functional reproductives produce a chemical signature that identifies their level of fertility (Keller and Nonacs, 1993; Vargo, 1999; Peeters and Liebig, 2009). When perceived by target individuals, these signals affect their reproductive development possibly by influencing the endocrine system (Danty et al., 1999; Grozinger and Robinson, 2007; Fussnecker et al., 2011). However, since the discovery more than 50 years ago of a queen honeybee substance (Butler et al., 1959), little progress has been made in the chemical identification of queen pheromones in other social insects, especially termites.

Approximately 2,600 species of termites have been described (Kambhampati and Eggleton, 2000). Seven families

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defined within Isoptera are classically grouped into the lower termites (all families except the Termitidae) and the higher termites (Termitidae). The former group includes termites with hindgut protozoa, whereas the family Termitidae contains protozoa-independent species. Neoteny is a widespread phenomenon in lower termites. The term neotenic reproductive refers to any termite reproductive that is not derived from an alate (Thorne, 1996). Neotenic reproductives differentiate within their natal colony, never dispersing to outbreed. These neotenic individuals can differentiate from either nymphs to become 'nymphoid' reproductives with wing pads, or from workers to become 'ergatoid' reproductives without wing pads. Neotenic reproductives have been reported in 61.7 % of lower termite genera but in only 13.4 % of higher termite genera (Termitidae) (Myles, 1999). Neotenics develop as replacement reproductives in response to orphaning or as supplementary reproductives even in the presence of functional reproductives. The mechanism controlling the decision between neotenic reproduction and other options within the network of developmental pathways remains unclear.

Two possible mechanisms may regulate how termite reproductives inhibit nestmate fertility. One is direct physical manipulation through agonistic interactions. Another is inhibition through pheromones. Little evidence exists to support the former hypothesis (Roisin, 1994). Based on observations of *Reticulitermes hesperus*, Pickens (1932) first hypothesized that an inhibiting secretion, produced by a queen, prevented the development of other reproductives in the colony. Shortly afterward, studies of *Zootermopsis* offered strong evidence for the existence of inhibitory pheromones (Castle, 1934; Light, 1944). Lüscher (1961) proposed the famous model of social control, which posits that reigning reproductives (kings and queens) inhibit the development of neotenics by inhibitory substances distributed among nestmates through mutual contact. The absence of this inhibition results in the formation of neotenics. Pheromonal inhibition has been repeatedly reported in the reproductive division of labor within termite colonies (Castle, 1934; Light and Weesner, 1951). However, the chemistry of a putative queen pheromone was not identified in any termite species until very recently.

Volatile or Non-volatile?

In terrestrial environments, chemosignals can be either volatile or non-volatile. Colony members can recognize reproductives by reproductive-specific "surface pheromones" through direct physical contact. In addition to non-volatile surface pheromones, the utilization of volatile signals enables members to recognize the presence of reproductives at a distance. Colony size can affect the form of such chemical messages. In small colonies, direct interactions result in reproductive hierarchies (Fletcher and Ross, 1985). For example, in single-site wood nesters, including the families Termopsidae and Kalotermitidae, whose entire lives are spent at a single wood dwelling that serves as both shelter and food, maximum colony size is relatively small, ranging from 600 to 8,000 (reviewed by Lenz, 1994). In such single-site nesting species with small colony size, inhibition depends on direct physical contact and not on volatile substances (Lüscher, 1952; Springhetti 1972), and even a brief absence of functional reproductives triggers neotenic development (e.g., 48 hr in *Zootermopsis angusticollis* [Stuart, 1979] and *Kalotermes flavicollis* [Kindl and Hrdy 2005]).

When the society becomes large, it would be impossible for all colony members to contact the reigning queens directly. Only a small percentage of the members, i.e., retinue workers and soldiers, contact the reproductives. Thus, selection then favors the evolution of volatile queen pheromones, which transmit queen messages to many more individuals at one time within the colony. Early studies have suggested the possible existence of volatile queen pheromones for both lower termites (Lüscher, 1952, 1961) and higher termites (Camazine et al., 2003). However, the existence of volatile pheromones does not preclude the existence of surface pheromones. Non-volatile cues on the body surfaces of queens are essential for queen recognition, even after the evolution of volatile queen pheromones. Therefore, relatively primitive termites that live in small colonies would likely only use surface pheromones, whereas species that form large colonies should utilize both non-volatile and volatile queen pheromones.

About a half-century ago, Edward O. Wilson had already noted the discrepancy in the number of studies of volatile and non-volatile pheromones (Wilson, 1965). For various technical reasons, volatile pheromones have been relatively easy to extract and bioassay. On the other hand, non-volatile surface pheromones have proven far more refractory. When removed from other surface odors or when masked by alien substrates added during conventional extraction techniques, surface pheromones no longer produce pheromonal effects. In addition, bioassays for surface pheromones are also extremely difficult even if a queen-specific compound is found. To test the pheromone activity of a surface pheromone, a successful bioassay requires such a good dummy queen as to be recognized as a true queen by colony members. When we identified the termite egg recognition pheromone, i.e., egg surface pheromone, dummy eggs composed of glass beads induced egg-protection behavior by workers, as did true eggs (Matsuura et al., 2007). This bioassay was successful for egg recognition because physical cues are very simple, as the dummy items need only have the same size and smooth spherical surface as eggs. In the case of queen surface pheromones, however, physical cues would be far more complicated. Bioassays might be nearly impossible if they required behavioral cues.

In many eusocial Hymenoptera, cuticular hydrocarbons provide information about the egg-laying ability of female reproductives (Peeters et al., 1999; Liebig et al., 2000; Cuvillier-Hot et al., 2001; Sledge et al., 2001; Hannonen et al., 2002; Heinze et al., 2002; Dietemann et al., 2003; Hartmann et al., 2005; Holman et al., 2010). Although the original function of the cuticular lipid layer of insects is to protect against pathogens and water loss, hydrocarbons also function as chemical messengers between and within species. Recently, two independent studies have observed cuticular hydrocarbon profiles specific to functional neotenics in the kalotermitid termite *Cryptotermes secundus* (Weil et al., 2009) and the termopsid termite *Zootermopsis nevadensis* (Liebig et al., 2009). In addition, Hanus et al. (2010) reported that functional reproductives have sex-specific proteinaceous compounds in three termite species, *Prorhinotermes simplex*, *Reticulitermes santonensis*, and *Kalotermes flavicollis*. These studies have proposed cuticular hydrocarbons (Liebig et al., 2009; Weil et al., 2009) and proteinaceous compounds (Hanus et al., 2010) as candidate non-volatile queen pheromones. Unfortunately, there has been no evidence that these chemicals act as queen recognition and/or inhibition signals in termites, primarily due to the technical difficulties of the bioassays described above. Future technical developments might overcome these difficulties and reveal the exact functions of these non-volatile compounds.

First Identification of Termite Queen Pheromone

Lower termites of the genus *Reticulitermes* make good subjects for identifying queen pheromones, as sufficient numbers of reproductive queens can be collected because the primary queen (alate-derived) can be replaced by many neotenic secondary queens (Thorne et al., 1999; Vargo and Husseneder, 2009). *Reticulitermes speratus* is the most common termite in Japan. Colonies are usually founded by one king and one queen. Upon the death of the primary queen or king, neotenic reproductives are produced. Mature field colonies usually have a single primary king and an average of 55.4 female neotenic reproductives, which are almost exclusively produced parthenogenetically by the original primary queen (Matsuura et al. 2009a). In addition, in natural colonies, neotenic reproductives differentiate exclusively from nymphs, never workers (N=3,029), as nymphs have priority in becoming neotenics in this species (Matsuura et al. 2009a, 2010; Matsuura, 2010).

To test for the existence of queen inhibitory pheromone, we first compared the number of replacement queens produced in groups containing nymphs, workers, and a reproductive queen and in similar groups without a queen. In these experiments, some secondary queens and kings were produced from nymphs (nymphoid queens and kings) but not from workers (ergatoid queens and kings). The differentiation of new nymphoid queens was greatly suppressed by the presence of a functional queen (Matsuura et al., 2010). In contrast, queen presence did not significantly affect the differentiation of nymphoid kings. In a second experiment, we similarly investigated the inhibitory power of reproductive queens, but we used experimental units containing only workers. Under such conditions, some workers differentiated into ergatoid secondary queens and males. As in the previous experiment, the presence of a fertile queen greatly suppressed the differentiation of new ergatoid queens but not ergatoid kings. Thus, queens exerted a strong sex-specific effect on the differentiation of neotenic reproductives regardless of developmental origin. Additional experiments in which queens were confined within double-mesh cages revealed that the inhibitory effect of queens was mediated by volatiles.

To identify the volatile inhibitory pheromone produced by queens, we collected a large number of *R. speratus* colonies and used the two largest for chemical analyses. Headspace-collected volatiles from 100 fully developed secondary queens were analyzed using gas chromatography followed by mass spectrometry (HS GC-MS). We found that the queen volatiles consist of an ester n-butyl-n-butyrate (nBnB; CAS No. 109-21-7) and an alcohol 2-methyl-1-butanol (2M1B; CAS No. 137-32-6) in a 2.14:1 ratio. In contrast to queens, workers and nymphs did not produce any nBnB or 2M1B.

In the course of our trial to analyze queen volatiles, we realized that these queen-specific volatiles are truly “honest” fertility signals. Soon after isolation from the nest, the physogastric queens maintained in Petri dishes with workers reduced oviposition, likely due to poor provisioning. After they ceased egg-laying, the queen-specific volatiles were no longer detectable (Matsuura et al., 2010). This result appears to support the hypothesis that queen pheromones act as honest signals of queen fertility (Keller and Nonacs, 1993).

To investigate the effect of nBnB and 2M1B on queen differentiation, we developed a new protocol for gradually and continuously exposing termites to volatile compounds. The compounds, once absorbed by an unglazed ceramic ball, slowly volatilize in an outer Petri dish and then enter an inner Petri dish through a small opening on the lid. A 2:1 blend of commercial nBnB and 2M1B, matching the naturally occurring ratio, strongly suppressed the differentiation of workers into ergatoid queens (Matsuura et al., 2010; Matsuura and Yamamoto, 2011), whereas the substances had no significant effect on the differentiation of ergatoid kings. In addition, each single compound alone had no effect on queen differentiation compared to a control treatment (Matsuura et al., 2010; Yamamoto et al., 2011).

In chemistry, a molecule that is non-superposable on its mirror image is considered to be chiral, and the two mirror images of a chiral molecule are called enantiomers. The relationships between absolute configuration and pheromone activity are diverse and complicated (reviewed by Mori, 1998, 2007). 2M1B is the first chiral molecule identified as a primer pheromone in social insects, which presents the intriguing question of whether enantiomeric composition plays a role in caste regulation. To determine whether the (*R*)/(*S*) configuration of 2M1B influences the inhibitory activity in *R. speratus*, the (*R*)- and (*S*)-enantiomers of 2M1B were synthesized, and the number of female neotenic reproductives were differentiated from workers under exposure to (*R*)-, (*S*)-, and the racemic mixture of 2M1B in combination with nBnB was compared. The (*R*)- and (*S*)-enantiomers and the racemic mixture of 2M1B showed significant inhibitory effects on the differentiation of new female neotenics in combination with nBnB, and no significant differences in inhibitory activity were observed among these treatments (Yamamoto et al., 2011). This suggests that termites recognize 2M1B as a queen signal, but they do not distinguish among the stereostructures of the enantiomers, although the absolute configuration of the naturally occurring queen pheromone remains to be identified.

Egg Volatiles as Attractant and Fertility Signal

In addition to queens themselves, the existence of brood (eggs and larvae) may act as a direct signal of queen fertility. In the honeybee *Apis mellifera*, old larvae provide the primary signals that prevent ovary activation in workers (Trouiller et al., 1991; Arnold et al., 1994; Mohammedi et al., 1998; Oldroyd et al., 2001; Pankiw and Garza, 2007; Maisonnasse et al., 2010), and in the ant *Pachycondyla apicalis*, larvae affect worker reproduction in queenless groups (Heinze et al., 1996). In the ant *Myrmica rubra*, queen-laid eggs inhibit worker ovary development (Brian and Rigby, 1978). A recent study of the ant *Camponotus floridanus* demonstrated that queen-derived hydrocarbon-labeled eggs inform workers of the presence of a fertile queen, thus suppressing worker reproduction (Endler et al., 2004).

To test whether eggs may also have an inhibitory effect in *R. speratus*, we compared the differentiation of neotenic queens in colonies without queens, in which eggs were either absent or continuously added at a rate of 100 or 20 eggs/day. Differentiation rates significantly differed between colonies receiving 100 eggs/day and colonies without eggs, whereas colonies receiving 20 eggs/day and colonies without eggs did not significantly differ (Matsuura et al., 2010). This indicates that not only queens themselves, but also eggs in sufficiently large numbers, have a strong inhibitory effect on the differentiation of neotenic queens in this termite.

Interestingly, we determined that the two volatiles, 2M1B and nBnB, in the queen pheromone are also emitted by eggs (Matsuura et al., 2010). Egg volatiles might be transferred from queens through egg marking or could be emitted by the eggs themselves. An antibacterial protein lysozyme, which also functions as an egg-recognition signal, is synthesized in eggs (Matsuura et al., 2007), implying that termite eggs are biosynthetically active. If the volatiles are transferred from queens to the egg surface, the volatiles should be detected even after the eggs are killed by freezing. However, frozen-killed eggs show no volatile emission, indicating that the volatiles are actively produced by the eggs themselves.

Why do termite eggs emit volatiles identical to the queen pheromone? We investigated whether the volatile components emitted by eggs had additional functions. Termite eggs cannot survive without protection by workers (Matsuura et al., 2000; Matsuura, 2006). Soon after being laid, eggs are carried into nursery chambers where they are groomed by workers and coated with saliva and antibiotic substances. Workers recognize eggs by morphological cues and a termite egg recognition pheromone (TERP) consisting of an antibacterial protein lysozyme (Matsuura et al., 2007) and a digestive β-glucosidase enzyme (Matsuura et al. 2009b). These two compounds can only be detected when workers directly touch the egg surface. Our preliminary experiments demonstrated that workers aggregated around egg piles confined in a stainless steel mesh cage, suggesting that workers are attracted to eggs by volatile cues. To determine whether the egg volatiles nBnB and 2M1B are used by workers to locate eggs, we conducted bioassays using dummy eggs made of glass beads. Dummy eggs coated with TERP together with nBnB and 2M1B were carried into egg piles at a significantly higher rate than dummy eggs without nBnB and 2M1B. Without TERP, dummy eggs were not recognized as eggs even when coated with nBnB and 2M1B, indicating that egg volatiles act as an attractant but not as a recognition pheromone.

Queen–Queen Interaction via Volatile Pheromone

The question of resource allocation has long been a core issue in the study of social insects. When and how resources are allocated to growth, maintenance, and reproduction determines colony survival and growth (Oster and Wilson, 1978). In eusocial insects, young broods need tending and nourishment by workers. Therefore, egg production must be adjusted in proportion to the capacity of the colony to rear the brood to maximize productivity (Tschinkel, 1988; Matsuura and Kobayashi, 2010). To accomplish optimal resource allocation to queens and the brood, colony members must adjust investment to queens in accordance with the current reproductive power of the queens. In the presence of multiple queens, regulation of colony-level egg production involves interactions among the queens. For example, Vargo (1992) demonstrated that queen-produced pheromones were involved in inhibition of the reproductive output of coexisting queens in the ant *Solenopsis invicta*.

To determine whether the queen pheromone influences the reproductive rate of queens in *R. speratus*, we compared the number of eggs produced by queens with and without exposure to an artificial queen pheromone. We kept either one or three mature female neotenic reproductives with 200 workers on a block of mixed sawdust food in a Petri dish and then compared the number of eggs produced between monogynous (single-queen) and polygynous (multiple-queen) groups. Exposure to the artificial queen pheromone significantly reduced the total number of eggs produced by the queens in each group, whereas no significant difference was observed in the total number of eggs produced between single-queen and three-queen groups (Yamamoto and Matsuura, 2011). The average number of eggs produced per queen in groups with multiple queens was significantly lower than in groups with a single queen. The number of eggs laid per queen was also reduced by exposure to the artificial queen pheromone. Hence, the total number of eggs produced in single-queen and multiple-queen groups did not significantly differ when these colonies had an equal number of workers, because the egg production of one queen negatively affected that of any other queens in a colony (Yamamoto and Matsuura, 2011). Reduced egg production under exposure to the artificial queen pheromone suggests that this mutual inhibition could be caused by the volatile chemical emitted by the queens and eggs.

We propose three possible mechanisms for this process: 1) Queens make a choice, whereby nourishment by workers is adjusted based on queen behavior such as begging frequency; 2) workers make a choice, whereby they control the amount of food supplied to each queen based on pheromonal information; or 3) the queen-produced pheromone acts directly on the queen's neuroendocrine system to affect egg production. Careful observation of queen feeding by workers with and without exposure to the queen pheromone might distinguish between hypotheses 1) and 2). Inactivation of the pheromone receptor of queens or workers may also be key to a better understanding of the regulatory mechanism.

Queen-Specific Volatile in a Higher Termite

To understand the evolution of queen pheromones and social regulation in termites, it is essential to compare pheromone compounds among diverse taxa. As the first step for a comparative study, we performed chemical analysis of the queen volatiles of the higher termite *Nasutitermes takasagoensis* (Termitidae), which is phylogenetically distant from *R. speratus*. The foraging behavior of *N. takasagoensis* is a separate type, whereas that of *R. speratus* is an intermediate type (Abe, 1987). Both species are parasitized by egg-mimicking fungi, although the parasitism evolved independently in each genus (Matsuura and Yashiro, 2010).

To determine the queen-specific volatile compounds in *N. takasagoensis*, headspace-collected volatiles from individual fully physogastric primary queens were analyzed using HS GC-MS. We identified a single queen-specific compound, phenylethanol (Himuro et al., 2011), which differed from the volatile queen-pheromone identified in *R. speratus*, suggesting that the chemical compositions of queen pheromones may vary greatly among termite taxa.

Phenylethanol is a common semiochemical that can be found in a broad range of insect species. It serves a variety of behavioral functions, for example, as a component of the aggregation pheromone in the cerambycid beetle *Megacyllene caryae* (Lacey et al., 2008), of the sex pheromone in male cabbage moths (Bestmann et al., 1977; Jacquin et al., 1991), and of the alarm pheromone in the ant *Crematogaster nigriceps* (Wood et al., 2002). Interestingly, phenylethanol is one of the queen-specific compounds in the European honeybee *Apis mellifera*, although its function is unknown (Gilley et al., 2006).

The exact functions of the queen-specific volatile of *N. takasagoensis* remain to be determined. Ergatoid reproductives reportedly develop upon the death of primary queens or after a reduction in the reproductive power of old primary queens in *N. takasagoensis* (Miura and Matsumoto, 1996), suggesting that pheromones produced by reigning queens inhibit the differentiation of new neotenic reproductives. The queen-specific volatile phenylethanol may function as a reproductive inhibitory pheromone in this termite. However, direct proof of reproductive inhibition is particularly difficult to obtain in higher termites for technical reasons. We were unable to apply the inhibition bioassay used for *R. speratus* to *N. takasagoensis*, as it was difficult to keep workers and nymphs alive after separation from the nest structure. Maintaining the nests in the laboratory also is very difficult. Therefore, another type of bioassay is needed to determine the function of queen-specific volatiles in higher termites. In the higher termite *Macrotermes subhyalinus*, queen volatiles serve as releaser pheromones for construction of the royal chamber by workers (Bruinsma, 1979). Such building behavior might be useful for developing a queen pheromone bioassay for higher termites.

Proximate Origin and Evolutionary Parsimony

As discussed in the review by Blum (1996), a wide variety of insects have adapted their own semiochemicals to subserve multiple functions in diverse contexts. Because the number of candidate compounds available to regulate multiple systems may be biosynthetically finite, strong selection favors the use of single natural products for many purposes. Secondary use of chemical compounds that have evolved for other primary functions occurs in various social insects (Blum and Brand, 1972; Turillazzi et al., 2006; Cremer et al., 2007). Identification of the termite egg-recognition pheromone elucidated such evolutionary parsimony (Matsuura et al., 2007, 2009b). The primary functions of the pheromone components lysozyme and β-glucosidase are as an antibacterial defense agent and a digestion enzyme, respectively. Termites have evolved to use the preexisting chemicals on the egg surface as egg-recognition signals, without the evolution of any additional specific chemical for this purpose.

Therefore, it is reasonable to hypothesize that the volatile compounds of termite queen pheromones, 2M1B and nBnB, would also have practical functions other than their pheromonal roles. The nesting and feeding ecology of termites expose colony members to a great variety of microbes including bacteria, fungi, protozoa, viruses, spirochetes, and nematodes (Rosengaus and Traniello 2001; Traniello et al., 2002). Therefore, one of the most important selection pressures on termites is how they cope with various microorganisms, resulting in the evolution of behavioral and physiological adaptations. Because an infection of a queen is fatal to colony survivorship, the queen is especially protected against incoming parasites (Cremer et al., 2007). The exudates of the physogastric queen of the termite *Termes redemanni* have known antimicrobial properties (Sannasi and Sundara Rajulu, 1967).

Recently, we determined that 2M1B and nBnB have inhibitory effects on the germination and mycelial growth of the "termite ball" fungus (K. Matsuura and T. Matsunaga, unpublished data). Termite ball is a sclerotium of an athelioid fungus (Basidiomycota, Agaricomycotina) of the genus *Fibularhizoctonia* (Matsuura et al., 2000; Yashiro and Matsuura, 2007; Matsuura and Yashiro, 2009). Termite balls mimic termite eggs and are thus tended by termites in the egg piles, whereby the fungus gains a competitor-free habitat in termite nests. Most termite balls are inhibited from germination in egg piles. However, if termite balls germinate in egg piles, the fungus consumes surrounding eggs (Matsuura et al., 2000; Matsuura, 2006). Therefore, an antifungal function of the egg volatiles seems reasonable. Interestingly, the inhibition activities of 2M1B and nBnB vary among strains of the fungus: 2M1B shows a stronger inhibitory effect than nBnB on one fungal strain, but the opposite is true of another strain. This result is indicative of an "arms race" between the parasitic fungus and termites and might explain why the queens and eggs emit multiple volatiles.

Future Perspective

To understand the evolution of multifunctional queen pheromones in termites, I propose two further directions that include 1) comparison of queen substances among a variety of species, and 2) identification of the molecular basis of pheromone activities. For the comparative approach, queen pheromone needs to be identified in many more species of many taxa. The difficulties both in collecting adequate queen substances and in testing the inhibitory activity have "concealed" queen pheromone. A sure way of obtaining a sufficient amount of queen volatiles is to collect physogastric queens from mature field colonies. It is important to note that volatiles need to be collected from queens soon after extraction from the nest (hopefully within 24 hours) because isolation from the colony reduces egg production and volatile emission very quickly. In the bioassay, it is essential to expose termites to the volatile compounds gradually and continuously. To this end we have developed a new protocol where the compounds once absorbed by an unglazed ceramic ball slowly volatilize in the outer Petri dish and then enter the inner Petri dish through a small opening on the lid (Matsuura et al., 2010). This device would be useful in testing the inhibitory activity of volatiles in a species in which neotenics easily develop after orphaning.

Identification of a queen pheromone that suppresses differentiation of new neotenic queens opens up exciting new possibilities to investigate how exocrine signals interact with the endocrine system to regulate caste differentiation. Recent studies have begun to shed light on the molecular basis for division of labor and caste determination in termites. The relationship between juvenile hormone (JH) synthesis and egg production has been demonstrated in *Zootermopsis angusticollis* (Greenberg and Tobe, 1985; Brent et al., 2005), *Z. nevadensis* (Miura et al. 2003), *R. flavipes* (Scharf et al., 2005), and *R. speratus* (Maekawa et al., 2010). A gene *Neofem*2, which is overexpressed in queens but not in kings and workers, is necessary for the queen to suppress worker reproduction in *Cryptotermes secundus* (Korb et al. 2009). Characterization of the pheromone receptors and their downstream targets, as well as the elucidation of pheromone biosynthesis, should provide important new insights into how reproductive and non-reproductive developmental pathways are regulated in termite colonies.

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Root Herbivore Effects on Aboveground Multitrophic Interactions: Patterns, Processes and Mechanisms

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Abstract In terrestrial food webs, the study of multitrophic interactions traditionally has focused on organisms that share a common domain, mainly above ground. In the last two decades, it has become clear that to further understand multitrophic interactions, the barrier between the belowground and aboveground domains has to be crossed. Belowground organisms that are intimately associated with the roots of terrestrial plants can influence the levels of primary and secondary chemistry and biomass of aboveground plant parts. These changes, in turn, influence the growth, development, and survival of aboveground insect herbivores. The discovery that soil organisms, which are usually out of sight and out of mind, can affect plant-herbivore interactions aboveground raised the question if and how higher trophic level organisms, such as carnivores, could be influenced. At present, the study of above-belowground interactions is evolving from interactions between organisms directly associated with the plant roots and shoots (e.g., root feeders - plant - foliar herbivores) to interactions involving members of higher trophic levels (e.g., parasitoids), as well as non-herbivorous organisms (e.g., decomposers, symbiotic plant mutualists, and pollinators). This multitrophic approach linking above- and belowground food webs aims at addressing interactions between plants, herbivores, and carnivores in a more realistic community setting. The ultimate goal is to understand the ecology and evolution of species in communities and, ultimately how community interactions contribute to the functioning of terrestrial ecosystems. Here, we summarize studies on the effects of root feeders on aboveground insect herbivores and parasitoids and discuss if there are common trends. We discuss the mechanisms that have been reported to mediate these effects, from changes in concentrations of plant nutritional quality and secondary chemistry to defense signaling. Finally, we discuss how the traditional framework of fixed paired combinations of root- and shoot-related organisms feeding on a common plant can be transformed into a more dynamic and realistic framework that incorporates community variation in species, densities, space and time, in order to gain further insight in this exciting and rapidly developing field.

Keywords Above-belowground interactions · Multitrophic interactions · Plant-insect interactions · Parasitoids · Plant defense

Introduction

A central subject in terrestrial ecology is to understand the driving forces underlying the assemblage and functioning of plant-based communities. Within this field, the study of plant-insect interactions has played a pivotal role. Plant-insect interaction studies traditionally have focused on organisms that share a common domain, mainly aboveground. Aboveground herbivorous insects are the most speciose animal group on earth, and the intricate interactions with their host plants have fascinated ecologists for decades. In response to herbivory, plants often are defended by the production of or increase in the production of secondary

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plant compounds, phytotoxins, which impact the herbivore's feeding activity and/or development. These plant defense responses often result in increased mortality, reduced growth rates and fitness of the attacker (Schoonhoven et al., 2005). Herbivorous insects, on the other hand, have evolved ways that detoxify such deleterious plant chemicals. Increased plant resistance in response to herbivory is called induced direct plant defense. Concentrations of plant defense compounds do not only occur locally in the leaf subjected to herbivory, but often increase in other leaves as well. Such a systemic response enables the protection of the still undamaged leaves from the herbivore. As a consequence, this response also can influence the performance of other organisms that are feeding from the same plant, but at other locations or later in time. In response to herbivory and egg deposition, plants also emit volatile secondary metabolites, which can be used by natural enemies of the herbivores, for example insect parasitoids, to locate their hosts (Dicke and Sabelis, 1988; Turlings et al., 1990; Vet and Dicke, 1992; De Moraes et al., 1998; Dicke, 1999; Fatouros et al., 2008). This response, known as induced indirect plant defense, is beneficial for parasitoids, because these detectable plant cues can indicate the presence of their 'hard to detect' hosts (Vet et al., 1991). The plants subsequently benefit from reduced levels of herbivory due to increased top-down control. The phytotoxins consumed by herbivores often accumulate in tissues such as fat body and hemolymph, and via this mechanism plants may also negatively affect the fitness of the developing parasitoid larvae that consume the host herbivore. This exemplifies how plant defenses can cascade up trophic chains in complex ways (Harvey et al., 2003). Because herbivore-induced direct and indirect plant defenses mediate interactions between species within and between trophic levels, across space and time, they are considered a central force in assembling plant-based communities (Kaplan and Denno, 2007).

In the field, plants also are exposed to belowground consumers. In many terrestrial ecosystems, root-feeding nematodes and insects are the dominant belowground attackers. In the early 1990's, Masters et al. (1993) were among the first to report that root feeders can significantly alter interactions between plants and aboveground herbivores. This awareness of plant-mediated above-belowground interactions has brought a new level of complexity to the field of plant-insect ecology (Van der Putten et al., 2001; Bardgett and Wardle, 2003; Wardle et al., 2004). Interactive effects between plant consumers across domains have been explained by various induced plant responses, and a number of more recent studies indicate that these interactions often are mediated by herbivore induced plant defenses (reviewed in Bezemer and van Dam, 2005; Kaplan et al., 2008a; van Dam, 2009). In the early 2000's, the question was raised whether and how changes within the plant induced by root herbivores could cascade up influencing parasitoids of foliar herbivores (Bezemer et al., 2005; Soler et al., 2005; White and Andow, 2006; Rasmann and Turlings, 2007). Other studies focussing on the effects of soil-dwelling plant mutualists have shown that, for example, arbuscular mycorrhizal fungi, plant growth-promoting rhizobacteria, and decomposers also can affect the growth and development of foliar herbivores and their level of parasitism (Masters et al., 2001; Van der Putten et al., 2001; Gange et al., 2003; Wurst and Jones, 2003; Guerrieri et al., 2004; Hempel et al., 2009; Pineda et al., 2010; 2012).

In the present review, we focus on the impact of root-feeding insects and nematodes on aboveground insect herbivores and their parasitoids; the effects of belowground symbionts are reviewed elsewhere in this issue (Jung et al. 2012, this issue). We first discuss the conceptual models that have been put forward to explain plant-mediated effects of root herbivores on aboveground insect herbivores; changes in plant nutritional quality and in secondary chemistry, from altered concentrations of foliar phytotoxins to defense signaling. The effects of root herbivory on higher trophic levels aboveground are comparatively less explored, and because general patterns cannot yet be drawn we discuss cases that exemplify the magnitude of these effects. We end by proposing that a way to advance this field is to study above-belowground interactions within a more dynamic and complex spatial-temporal approach that includes insect mobility and spatial and temporal aspects in experimental designs. A new approach that goes beyond the relatively static interactions between pairs of organisms forced to feed on the same plant at a single density and time.

Impact of Root-Feeding Insects on Foliar Herbivores

Quantitative reviews show that in the vast majority of cases, insect herbivores that feed from the same plant affect each other negatively (Denno et al., 1995). These plant-mediated competitive interactions often are caused by increases in secondary plant compounds induced by the initial attacking species that negatively affect the subsequent species (Kaplan and Denno, 2007). In Fig. 1, we summarize the main patterns and mechanisms that have been proposed to explain the, positive and negative, effects that root-feeding insects can have on the survival, fecundity, growth and/or development of aboveground insect herbivores. One of the earliest aboveground-belowground studies reported a positive effect of root-feeding insects on the performance of aboveground aphids, and attributed this facilitation to an improvement in shoot nutritional quality measured as increases in total soluble nitrogen (Gange and Brown, 1989). Later studies further confirmed that aphids perform better when feeding on plants previously colonized by root-

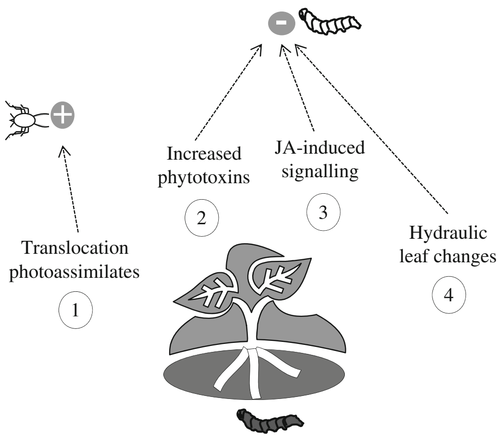

Fig. 1 *Plant-mediated effects of root-feeding insects on aboveground leaf chewers and phloem feeders.* The aphid (left) represents aboveground phloem feeders, and the white caterpillar (right) represents leaf chewers. The grey caterpillar represents root-feeding insects. Effects of root herbivory can be positive (+) or negative (−) for overall aboveground insect performance, relative to insects on undamaged plants. Mechanisms that have been put forward to explain these plant-mediated effects are induced changes in shoot nutritional quality (1), shoot secondary chemistry (2 and 3), and hydraulic leaf changes (4). Numbers indicate each of the proposed hypotheses discussed in the text

feeding insects compared to uninfested plants (Moran and Whitham, 1990; Masters and Brown, 1992). Based on these results, Masters et al. (1993) proposed the first mechanistic hypothesis linking spatially separated herbivores, the 'Stress Response Hypothesis' (Fig. 1, ①). According to this hypothesis, the capacity of roots to acquire water and nutrients from the soil is constrained due to removal of root tissue. This creates an effect within the plant similar to water stress, leading to the accumulation of soluble nitrogen and carbon in the foliage, facilitating the growth and development of the herbivores. This hypothesis has been derived from the 'Plant Stress Hypothesis', which predicts that plants subjected to non-extreme abiotic stress, for example water limitation, shading or pollution, become more susceptible to herbivores due to a temporal increase in the amount of soluble nitrogen that is mobilized from the site of attack to sites of storage and new growth (White, 1984). More recent studies that also observed positive effects of root herbivory on aphid performance, did not find significant differences in concentrations of soluble nitrogen in plants with or without root-feeding insects (Johnson et al., 2009). It is noteworthy that during the last one or two decades various meta-analyses have shown that water stress in plants frequently does not lead to increased performance of aphids (Koricheva et al., 1998; Huberty and Denno, 2004), which further challenges this hypothesis.

Other studies that have examined the effects of root herbivores on aboveground leaf chewers have reported negative effects, showing that besides facilitation, plant-mediated competition also is common in aboveground-belowground interactions (Tindall and Stout, 2001; Bezemer et al., 2003; van Dam et al., 2003, 2005; Soler et al., 2005; Staley et al., 2007). The frequently observed negative impact of root herbivory on leaf chewer fitness has been explained by the 'Defense Induction Hypothesis' (Bezemer et al., 2003). This hypothesis states that above- and belowground insect herbivores influence each other via induced changes in secondary plant compounds (Fig. 1, ②). Insects that feed from the phloem are less exposed to secondary plant compounds, since phytotoxins generally are stored in cells (Larsson, 1989). This can explain why aboveground aphids usually are not negatively affected by root herbivory. In this view, root-chewing insects induce an increase in foliar secondary plant compounds, which negatively affects the performance of leaf chewers without affecting phloem feeders (reviewed in Bezemer and van Dam, 2005; Johnson et al., 2008; Kaplan et al., 2008a; van Dam and Heil, 2011).

There has been a significant development in the understanding of the molecular mechanisms underlying local and systemic induced plant defenses triggered by pathogens and insects aboveground (Kessler and Baldwin, 2002; Zheng and Dicke, 2008; Pieterse et al., 2009). This has enabled the exploration of induced plant defenses beyond measuring changes in nutrients and phytotoxins, thus providing a basis to mechanistically understand plant-mediated interactions. Generally, leaf-chewing insects such as caterpillars cause a response in the plant that triggers the jasmonic acid (JA) signaling pathway, while phloem-feeding insects such as aphids induce the salicylic acid (SA) signaling pathway. Although the majority of studies have focused on signaling responses in the foliage in response to shoot attack, these responses also occur in the roots (reviewed in Erb et al., 2009a). It has been shown that jasmonates can be transported from shoots to roots (Baldwin et al., 1994), showing how long distance defense signaling can occur across roots and shoots. The transport of jasmonates from roots to shoots can explain why root-feeding insects may negatively impact the performance of foliar insect herbivores, because JA in the roots is transported to/activated in the shoots (Fig. 1, ③).

Jasmonic acid and salicylic acid often act antagonistically, and increases in the levels of one of the phytohormones can interfere with the activity of other phytohormones (Pieterse and van Loon, 1999; Engelberth et al., 2001; Kessler and Baldwin, 2002; Koornneef et al., 2008; but see e.g., Schenk et al., 2000; Van Wees et al., 2000 that report synergistic interactions). If this so-called cross-talk between pathways (Pieterse et al., 2009) also occurs across plant organs, root herbivory can cause a reduction in SA-related defenses in the foliage by inducing JA-related defenses as proposed by Van der Putten et al. (2001). This can provide an alternative explanation for the frequently observed increased performance of phloem feeders on plants previously attacked by root-feeding insects. However, in *Zea mays* (maize) plants, neither JA nor SA were found to be induced in the shoots by the rootworm *Diabrotica virgifera* (Erb et al., 2009b). Interestingly, leaves of root-infested maize plants had reduced leaf water contents and increased levels of abscisic acid (ABA) (Erb et al., 2011a).

Reduced resistance to leaf chewers has been reported on ABA-deficient plants (Thaler and Bostock, 2004; Bodenhausen and Reymond, 2007), leading the authors to hypothesize that, in *Z. mays*, increased resistance to leaf chewers in plants with root herbivory is due to induced ABA signaling and/or hydraulic changes in the leaves (Erb et al., 2011a). Abscisic acid is involved in a number of physiological adaptations of plants to drought stress, and it can act as a chemical signal that controls the opening and closing of stomata. It might be difficult then to disentangle the effects of changes in ABA and leaf water content on foliar herbivores. Interestingly, the negative effects on the leaf chewer were still observed after ABA signaling was inhibited. More studies that explore defense signaling that cross the border between the below- and aboveground domains are needed to understand the mechanistic basis that mediate these interactions (Erb et al., 2009a).

Knowledge about the molecular mechanisms underlying plant defenses is derived from a limited number of model plants species from genetic and molecular biology (Felton and Korth, 2000; Stout et al., 2006; Wang et al., 2008; but see Wu and Baldwin, 2010; Broekgaarden et al., 2010), and often herbivory is simulated by using exogenous applications of JA and SA (e.g., Spoel et al., 2003; Koornneef et al., 2008; Leon-Reyes et al., 2010; but see e.g., Kessler et al., 2004). Consequently, extrapolations into ecologically representative scenarios have to be taken with caution. Studies with natural communities are needed to determine the full ecological and evolutionary consequences of above-belowground multitrophic interactions.

Impact of Root-Feeding Nematodes on Foliar Herbivores

Root-feeding nematodes are dominant belowground herbivores and important pests worldwide. They are the main group of root herbivores in temperate grasslands and their feeding activities can affect aboveground plant size and nutritional quality (Stanton, 1988). The impact of root-feeding nematodes on aboveground insects has been less well-studied than the effects of root-feeding insects. However, an increasing number of studies are showing that root-feeding nematodes also can influence aboveground insects via their effects on the shared host plant (e.g., Bezemer et al., 2005; Kaplan et al., 2011). In Fig. 2, we summarize the most commonly observed effects, and discuss potential mechanisms to explain these linkages.

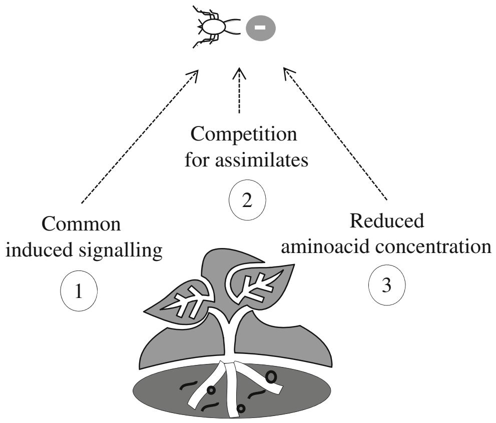

Fig. 2 *Plant-mediated effects of root-feeding nematodes on aboveground aphids.* The aphid represents aboveground phloem feeders, and the black circles and curved lines represent ecto- and migratory endoparasitic nematodes and root-knot or cyst-forming nematodes, respectively. Effects of herbivory by nematodes on aphid fitness are mostly negative (−) relative to that on undamaged plants. Mechanisms that have been put forward to explain these negative effects are induction of common defense signaling (1), competition for assimilates in the phloem (2), and reduced amino acid concentration in the phloem (3). Numbers indicate each of the proposed hypotheses discussed below

In contrast to root feeding by insects, which often facilitate the growth and development of aphids, studies on feeding by nematodes consistently report negative effects on aphid performance (Bezemer et al., 2005; Wurst and Van der Putten, 2007; Kaplan et al., 2009, 2011; Hol et al., 2010; Vandegehuchte et al., 2010; Kabouw et al., 2011). Nematode-caterpillar interactions are less well-studied, and positive (Alston et al., 1991; Kaplan et al., 2008b), neutral (Wurst and Van der Putten, 2007), and negative effects (van Dam et al., 2005) have been reported. We will, therefore, focus on the mechanisms that have been proposed to link the consistent negative impact of nematodes on aphid fitness. The first proposed explanation was that nematodes and phloem feeders trigger a common defense signaling pathway (Kaplan et al., 2009). This hypothesis is based on studies that showed that in Solanaceae, the defense gene Mi-1 mediates resistance to both root-knot nematodes and aphids (Li et al., 2006; Bhattarai et al., 2007). Thus, aboveground phloem feeders and root-feeding nematodes might be inducing similar defense pathways in plants (Fig. 2, ①). Subsequent studies have shown that although Mi-1 mediates resistance to both nematodes and phloem feeders/sap suckers, it is involved in the activation of distinct signaling pathways. Therefore, the Mi-1 defense gene may contribute differently to the resistance to aphids and nematodes (Mantelin et al., 2011). There is no empirical evidence yet that links the reduced performance of phloem feeders on plants exposed to nematodes with changes in levels of phytohormones or defense marker genes.

More recently, Kaplan et al. (2011) empirically tested the 'Sink Competition Hypothesis', which proposes that aboveground phloem feeders and root-feeding nematodes compete for assimilates in the phloem. Root-knot nematodes and aphids feed from vascular tissues and attract photoassimilates to their feeding site. Therefore, the pressure-driven transport in the phloem sieve elements can be re-directed towards root-feeding nematodes or aphids, and thus both can act as a nutrient sink for the plant (Guerrieri and Digilio, 2008). Thus, when nematodes colonize the roots of the plant earlier than aphids, the sink created by nematodes in the roots may compete with the subsequent sink that aphids will initiate in the shoots (Fig. 2, ②). Empirical evidence for this potential mechanism is lacking (but see Inbar et al., 1995; Larson and Whitham, 1997 for evidence supporting the hypothesis in aboveground plant-herbivore interactions). Especially cyst- or gall-forming species are able to feed from the phloem, which makes them potential competitors of aphids. It is noteworthy that aphids also perform suboptimally on plants infested by migratory endoparasitic species that do not create nutrient sinks within the plant (e.g., Wurst and Van der Putten, 2007). The concentration of amino acids in the phloem of plants infested by root-feeding nematodes also has been reported to be lower than on plants without nematodes, and this change correlated with the reduced aphid fitness that was observed (Bezemer et al., 2005). More studies are needed to confirm how widespread this mechanism is.

Root Feeders and Aboveground Parasitoids: Potential Interactions

Interactions via Changes in Herbivore Induced Plant Volatiles In the early 2000's, the question was raised whether soil-dwelling organisms also could affect parasitoids of aboveground herbivores. The first studies focused on parasitoid host-plant preferences, and all reported that the level of attraction of female parasitoids was increased when plants were exposed to soil-dwelling organisms, independently of the soil functional group triggering the effect. Therefore, it was proposed initially that soil organisms, independent of whether they were root antagonists or plant beneficials, would all benefit host-parasitoid interactions (e.g., Masters et al., 2001; Gange et al., 2003; Wurst and Jones, 2003; Guerrieri et al., 2004). However, a potential mechanism responsible for the increase in host plant preference was not provided in these studies. Considering that in aboveground systems, parasitoid host-searching is guided primarily by volatile cues that are produced by the host-infested plant (Dicke, et al., 1990; Turlings, et al., 1990; Vet and Dicke, 1992), herbivore-induced plant volatiles were a primary candidate to test. Subsequent studies have shown that the composition of the volatile blend induced by foliar herbivores can be affected by root-feeding insects. The result is that the plant becomes less attractive to female parasitoids foraging for hosts (Rasmann and Turlings, 2007; Soler et al., 2007a). In these studies, root-feeding by insects clearly interfered with host-parasitoid interactions. Other studies also have shown that volatiles emitted by plants exposed to both foliar- and root-feeding insects can be quantitatively and qualitatively different from blends emitted by plants exposed to each herbivore in isolation (Olson et al., 2008; Pierre et al., 2011). It is well-established that specialist parasitoids can distinguish between plants attacked by their hosts and plants attacked by non-hosts by exploiting differences in induced plant volatiles (de Moraes et al., 1998). It is less clear, however, what can happen when the same plant is exposed to multiple host and non-host herbivores of the parasitoid (but see Shiojiri et al., 2001, 2002; Vos et al., 2001; Rodriguez-Soana et al., 2002; 2005; Zhang et al., 2009; Dicke et al., 2009; Erb et al., 2010), especially when these herbivores feed from roots and shoots.

Interactions via Changes in Host Quality and Consequences for Parasitoid Behaviour Parasitoid larvae are highly susceptible to changes in the quality of the internal biochemical environment provided by their hosts, and thus are tightly linked to host development (Harvey, 2005). As root herbivores can influence the growth and development of aboveground insect herbivores via induced changes in foliar secondary chemistry, these effects also could affect the developing parasitoid larvae. A number of studies have shown that root herbivore effects can even be stronger for the developing parasitoid larvae than for the herbivore itself (Bezemer et al., 2005; Soler et al., 2005, but see Kabouw et al., 2011 where no effects were observed). These effects can cascade up to at least the fourth trophic level influencing hyperparasitoid fitness (Soler et al., 2005).

Unlike predators, which frequently consume multiple prey individuals, the resources available for parasitoid development are restricted to a single host. Consequently, parasitoids are under strong selection pressure to optimize usage and disposal of these limited resources (reviewed in Harvey, 2005). Optimal foraging theory predicts that carnivores choose to attack host/prey species that are most rewarding for them in terms of their fitness (Krebs and Davies, 1984). Similarly, within a host species, parasitoid females are expected to select the most profitable individuals that maximize their fitness (Godfray, 1994). Since the adequacy of foraging choices of parasitoids is linked directly with their reproductive success, females can be expected to select in favor or against hosts feeding on plants already infested by root herbivores, depending on how root herbivory affects the performance of the parasitoid. Most studies that link above-belowground multitrophic interactions address either effects on parasitoid attraction or changes in plant volatiles but not both. Therefore, it remains unclear how common it is that root herbivory affects aboveground host-parasitoid interactions by changes in plant volatile emission. In Table 1, we summarize studies that have addressed these aspects.

In Fig. 3, we summarize case studies that provide support for the hypothesis that the degree of preference of female parasitoids for hosts feeding on plants already infested by root herbivores will depend on how root herbivory affects the performance of their offspring (Soler et al., 2005, 2007a). *Cotesia glomerata* females parasitized significantly more *Pieris brassicae* hosts on *Brassica nigra* plants without than with the root herbivore *Delia radicum* (Fig. 3a). Parasitoids also developed significantly better on hosts that were feeding on plants without root herbivory (Fig. 3a). In the presence of root herbivory, the amount of sinigrin, which represented 99% of the total glucosinolate contents in the shoots of *B. nigra*, was significantly higher (Fig. 3b). The suboptimal parasitoid performance in root-infested plants was attributed to the increased sinigrin concentration in shoots of plants with root herbivores. This behavior shows a clear preference-performance linkage for the parasitoid that will enhance the performance of its offspring. The volatile blends emitted by undamaged plants, by plants damaged by *Pieris brassicae* (the leaf-chewing host of the parasitoid), by plants exposed to *Delia radicum* (the root herbivore), and by plants exposed to both types of herbivory differed significantly (Fig. 3c). Plants exposed to the leaf chewer were characterized by high levels of beta-farnesene and dimethylnonatriene, which are volatile compounds reported to act as attractants for herbivorous and carnivorous insects (Dicke et al., 1990; Fukushima et al., 2002; Ansebo et al., 2005). In contrast, plants exposed to root herbivory were characterized by high amounts of sulphides, such as dimethyl disulfide and dimethyl trisulfide, which act as repellents/toxins to insects (Dugravot et al., 2004). The reduced preference of female parasitoids for hosts feeding on plants colonized by root-feeding insects may be attributed to the relatively high levels of repellents and low levels of attractants that root and shoot co-infested plants emit compared to conspecific plants with only hosts. Taken together, these results suggest that root-damaged plants convey chemical information that aboveground parasitoids can use to optimize oviposition decisions (but see Olson et al., 2008). This expectation is confirmed by these studies, but support for this hypothesis remains scarce.

Innate responses of foraging parasitoids to plant odors can change with experience, leading to local or temporary specialization and enhancement of foraging success (Turlings et al., 1990; Vet et al., 1995). Parasitoids have the ability to learn to distinguish between volatile blends emitted by plants infested by their hosts *versus* plants infested with their hosts and root-feeding insects (Rasmann and Turlings, 2007). Therefore, they could regain attraction for hosts feeding on root-infested plants with experience (Rasmann and Turlings, 2007). Yet, the effects of parasitoid learning in this process need to be explored. The role of parasitoid learning in dealing with natural variation in plant and host quality and plant volatiles induced by root herbivory remains largely unstudied.

Incorporating Community Variation in Species, Densities, Space, and Time

Thus far, the majority of above-belowground interaction studies that involve plants, insects, mutualistic symbionts, and natural enemies have encompassed relatively little variation in number of players and in environmental conditions. Here, we review studies that are extending this scope by bringing in effects of time, space, behavior, and habitat conditions. We identify this as the direction of future studies in the area of above-belowground multitrophic interactions.

Time of Arrival of Root and Shoot Herbivores The sequence of arrival of above- and belowground herbivores on a plant can greatly affect the outcome of the interaction (Maron, 1998; Blossey and Hunt-Joshi, 2003). The leaf chewer *Spodoptera fugiperda*, for example, had a significant negative effect on the colonization of the root chewer *Diabrotica virgifera* when first colonizing the plant, but the aboveground herbivore did not influence the performance of the root feeder when arriving later than the root herbivore (Erb et al., 2011b). The sequence of arrival also has been shown to be an important determinant of plant responses at the gene level. Transcriptional changes, for example, have been shown to differ significantly for sequential and simultaneous attack of aboveground leaf chewers and phloem feeders (Voelckel and Baldwin, 2004). Similarly, the expression of SA- and JA-related genes has been found to differ in response to individual and simultaneous shoot attack by insect herbivores from contrasting feeding-guilds (Zhang et al., 2009; Soler et al., 2012). Aboveground insect herbivores that feed on a plant already infested by root feeders are expected to be inevitably confronted with higher levels of phytotoxins, and thus potential fitness costs (Bezemer and van Dam, 2005). This idea is based on studies with *Gossypium herbaceum*, cotton plants, that showed that in response to root herbivory levels of secondary compounds increased along the entire shoot (Bezemer et al., 2004). However, it is not clear how widespread this response can be. For example, a subsequent study in which *B. nigra* plants were exposed to root herbivory showed that levels of secondary compounds were increased only in young leaves in response to root feeding, but that they did not change in mature and old leaves (Soler et al., 2005). More studies that record changes in secondary chemistry in response to root herbivory that compare both young and old leaves are needed to determine how common this phenomenon is.

Spatial Distribution of Root Feeders Besides the mere presence or absence of root feeders on the plant, the spatial distribution of root-infested plants in a habitat can be of crucial importance. Evidence for this assumption is provided by a field study where the specialist aphid *Brevicoryne brassicae* preferred to feed and reproduce on *B. nigra* plants without root herbivores over plants infested by the root herbivore *D.*

Table 1 Effects of root feeding insects (a) and nematodes (b) on parasitoid performance, behavior, and/or changes in plant volatiles. Rch: root-chewer, rk: root-knot, sp: seed predator, lch: leaf chewer, and pf: phloem feeder

Root herbivores	Plant species	Foliar herbivores	Parasitoids	Performance-related effects	Behavioural-related effects	HIPV changes	Reference
(a) Insects							
General insects	*Cirisum palustre*	*Terellia ruficauda* (sp)	*Pteromalus elevatus* *Torymus chloromerus*		Increased population abundance		Masters et al., 2001
Delia radicum (rch)	*Brassica nigra*	*Pieris brassicae* (lch)	*Cotesia glomerata*	Reduced size Longer development			Soler et al., 2005
Diabrotica virgifera (rch)	*Zea mays*	*Ostrinia nubilalis* (lch)	*Macrocentrus grandii*		Reduced population abundance		White and Andow, 2006
Delia radicum (rch)	*Brassica nigra*	*Pieris brassicae* (lch)	*Cotesia glomerata*		Attraction attenuation	More repellent+less attractants	Soler et al., 2007a
Delia radicum (rch)	*Brassica nigra*	*Pieris brassicae* (lch)	*Cotesia glomerata*		Reduced searching efficiency		Soler et al., 2007b
Diabrotica virgifera (rch)	*Zea mays*	*Spodoptera littoralis* (lch)	*Cotesia marginiventris*		Attraction attenuation Associative learning		Rasmann and Turlings, 2007
Agriotes spp. (rch)	*Plantago lanceolata*	None	None			No observed effect	Wurst et al., 2008
Delia radicum (rch)	*Brassica rapa*	*Pieris brassicae* (lch)	None			Qualitative+quantitative differences	Pierre et al., 2011
(b) Nematodes							
General Nematodes	*Agrostis capillaris* *Anthoxanthum odoratum*	*Rhopalosiphum padi* (pf)	*Aphidius colemani*	Reduced mortality			Bezemer et al., 2005
Meloidogyne incognita (rk)	*Gossypium* spp.	*Heliocoverpa zea* (lch)	*Microplitis croceipes*		No observed effect	Increased levels	Olson et al., 2008
General nematdes	*Brassica oleracea*	*Brevicoryne brassicae* (pf)	*Diaeretiella rapae*		No observed effect		Kabouw et al., 2011

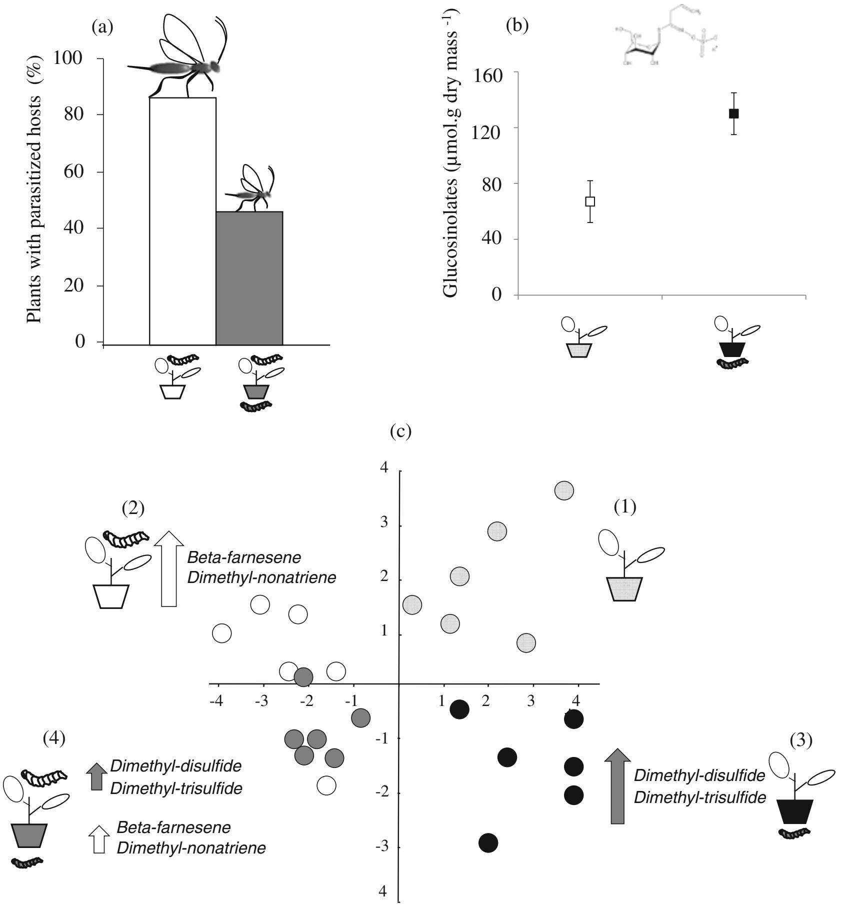

Fig. 3 *Root-feeding insects and aboveground parasitoids. A case study.* **a** Percentage of *Brassica nigra* plants with foliar-feeding *Pieris brassicae* hosts selected for oviposition by females of the parasitoid *Cotesia glomerata*. The size of the parasitoid reflects its relative performance on plants without (*white bars*) and with (*grey bars*) *Delia radicum* root-feeding larvae. **b** Glucosinolate (*sinigrin*) level in young leaves of *B. nigra* plants (*white dotted squares*) and plants infested by *D. radicum* (*grey squares*). **c** Canonical discriminant plot showing sample scores based on volatile blends of *B. nigra* plants (1) without herbivores (2) with *Pieris brassicae* larvae, (3) with *Delia radicum* larvae and (4) with both herbivores. Each circle represents a sampled plant. Beta-farnesene and dimethyl-nonatriene are known attractant compounds (*white arrows*) for insect parasitoids, while sulfides are known repellent volatiles (*grey arrows*) for insects; the size of the arrows represents the relative amount of the compounds in the blends of the plants with root- and foliar-feeding insects. Summary from R. Soler PhD Thesis, Netherlands Institute of Ecology, 2007 (reprints of the thesis can be requested by e-mail)

radicum. This preference was observed only when plants with root herbivores were grouped in clusters. When the plants with and without root herbivores were placed in a mixed design, aphids no longer differentiated (Soler et al., 2009). This shows that the spatial arrangement of root herbivores in the field also can be an important factor determining the amount of aboveground herbivory. However, as discussed in the previous section, it remains unknown whether root feeders uniformly influence the secondary chemistry of the entire shoot or if these changes are restricted to certain parts of the shoot. In response to aboveground insects, for example, phytotoxins often increase in certain tissues, e.g., young leaves, rather than uniformly along the shoot, thus allowing secondary attackers to scape potential fitness costs by avoiding feeding on theses leaves (Stout et al., 1996). When root induced plant responses are expressed only in certain parts of the shoot, only the aboveground herbivores that feed on these parts are expected to be influenced by root feeders (Kaplan et al., 2008c).

Herbivore and Parasitoid Preferences Most above-belowground studies are based on non-choice experiments where the survival, growth, and development of caterpillars or aphids on plants with or without root herbivores are compared. Foliar herbivores, however, can precisely select plants for oviposition and feeding. Where free choices can be made, aboveground insect herbivores can avoid or prefer plants that are already colonized by root feeders. Optimal oviposition theory predicts that females of herbivorous arthropods with offspring with limited mobility, such as butterflies, will evolve to select those host plants for oviposition on which their offspring perform best thus maximizing their fitness (Jaenike, 1990). Considering that plants attacked by root-feeding insects often represent a suboptimal food source for leaf chewers, butterflies should avoid plants with root herbivores and select uninfested conspecifics if these represent fitness costs (Soler et al., 2010). When such avoidance occurs, this also will be beneficial for the plant by reducing the probability of root-damaged plants being simultaneously attacked belowground and aboveground. The same approach might apply belowground, and there are studies, for example on root-feeding nematodes, where the presence of potential enemies may direct attackers away from potential feeding sites (Piskiewicz et al., 2009).

Adding effects on the reduced preferences that natural enemies of herbivores can show for hosts feeding on plants also attacked by root herbivores (Rasmann and Turlings, 2007; Soler et al., 2007a) will show the complex dimensions of the ecological 'dilemma' for leaf-chewing insects with respect to root-infested host plants. The evolutionary choice would be between growing more slowly and/or attaining a smaller size but benefitting from a smaller probability of being found by natural enemies on root-infested plants, or optimizing performance at the cost of running a higher risk of parasitism or predation on root-uninfested healthy plants. From the plant's point of view, the benefits of acting as a communication channel between root- and foliar-feeding herbivores that attenuates simultaneous infestations is then counterbalanced by interferences with the indirect defense system of the plant that reduces the attraction of natural enemies of the herbivore. If and how above- and belowground herbivores may integrate all this information in their "decision-making" remains to be elucidated.

Parasitoids and Effects Through Changes in the Habitat Interactions between root feeders and parasitoids are not restricted to interactions on a single plant. For example, root herbivores can influence host-parasitoid interactions aboveground via their effects on changes in the structure of the plants. In *Z. mays*, the percentage of parasitism of the European corn borer, *Ostrinia nubilalis*, by its specialist parasitoid *Macrocentrus grandii* was significantly reduced in the presence of the corn rootworm *Diabrotica virgifera* in the habitat (White and Andow, 2006). Plant height and density were reduced in habitats where the rootworm was present, resulting in more open habitats that are less preferred by female parasitoids of this species. Interestingly, this positive indirect interaction, known as associational resistance, in which one species gains protection from its consumer by association with a competitor, has been widely documented in plants (Andow, 1991), but not among insects. Root herbivores also can influence host-parasitoid interactions aboveground via changes in the quality of the surrounding environment triggered by belowground insects. Females of the parasitoid *Cotesia glomerata* found their hosts on focal plants much faster in situations when neighboring plants were exposed to root herbivory, than when neighboring plants were kept undamaged (Soler et al., 2007b). In that study, the microhabitat was composed of root-damaged and root-undamaged plants of the same species that all had similar size and height, which minimizes the influence of physical plant characteristics on the foraging wasps (McCann et al., 1998; Gols et al., 2005).

Plant-Mediated Aboveground-Belowground Interactions in the Field A number of studies have shown that the abundance or preference of aboveground organisms, such as herbivores, pollinators, predators, or parasitoids, on plants growing in natural or agricultural systems can be affected by whether the plant is also exposed to root herbivory (e.g., Masters, 1995; Poveda et al., 2003; Hunt-Joshi and Blossey, 2005; Staley et al., 2007; Wurst et al., 2008; Kaplan et al., 2009; Soler et al., 2009). Most of these studies have used potted plants with or without root herbivory that are placed in the field (e.g., Poveda et al., 2003; Wurst et al., 2008; Soler et al., 2009). However, several studies have manipulated aboveground and belowground herbivory in the field that show that root herbivory by insects or nematodes can affect aboveground multitrophic interactions under natural conditions (e.g., Blossey and Hunt-Joshi, 2003; White and Andow, 2006; Kaplan et al., 2009), while others have not detected a significant effect (Hladun and Adler, 2009; Hong et al., 2011; Heeren et al., 2012). Interestingly, two recent independent studies report that there are no significant interactions between soybean cyst nematodes and aphids in soybean fields (Hong et al., 2011; Heeren et al., 2012). In contrast, greenhouse studies with soybean plants have reported that the performance of soybean aphids is significantly influenced by cyst nematodes (e.g., Hong et al., 2010). These results indicate that care needs to be taken when extrapolating results from greenhouse and common garden experiments to real field situations, and emphasize the urgent need for more realistic above-belowground studies.

Belowground Influences of Aboveground Induced Defenses in the Field Another issue that remains largely unresolved is how important the effects of root herbivory on aboveground induced plant defense responses are for plants that are growing in the field and are interacting with multiple antagonists, mutualists, decomposers, and other plants simultaneously. Most field studies that examine root herbivore effects on aboveground plant-insect interactions do not report effects on secondary plant compounds or emission of volatiles. However, a recent study by Megias and Muller (2010) shows that exposure to root herbivory in field-grown brassicaceous plants (*Moricandia moricandioides*) led to significant changes in aboveground glucosinolate profiles, and that these differences correlate with changes in the composition of the aboveground food web on these plants. This study shows clearly that root induced changes in aboveground plant secondary compounds can be of significant importance in the field. Similarly, Hladun and Adler (2009) showed that *Cucurbita moschata* plants, butternut squash, grown in the field had increased floral nectar concentrations when exposed to root herbivory. This can subsequently affect pollinators, but also parasitoids and predators in the field. As there is now a considerable number of studies that have shown that levels of parasitism and predator abundance in the field can be affected by root herbivory (e.g., Masters et al., 2001; White and Andow, 2006; Soler et al., 2009), it is quite possible that root herbivory indeed affects aboveground indirect induced defense responses in the field. Further field-based studies are needed in order to determine how these interactions can influence, or are influenced by, species diversity and community structure. How important indirect plant defense responses can be in the field (Obermaier et al., 2008), and how this is affected by root herbivory remains to be explored.

Concluding Remarks

It is evident that root feeders can be important players in aboveground plant-based communities, via their effects on direct and indirect defenses of plant shoots that can cascade up to at least the fourth trophic level. Knowing this, the new challenge is to study above-belowground interactions under more realistic conditions. This will bring us closer to the detection of mechanisms with evolutionary potential and patterns that can be used in practice, for example when attempting to enhance sustainable pest control. It is puzzling why root-feeding insects and nematodes are still playing a minor role in the studies of contemporary community, behavioral, chemical, and molecular ecology. Currently, the notion of 'out of sight, out of mind' is no longer a valid argument for leaving out root feeders!

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REVIEW ARTICLE

The Chemical Ecology of Soil Organic Matter Molecular Constituents

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Abstract Soil organic matter (OM) contains vast stores of carbon, and directly supports microbial, plant, and animal life by retaining essential nutrients and water in the soil. Soil OM plays important roles in biological, chemical, and physical processes within the soil, and arguably plays a major role in maintaining long-term ecological stability in a changing world. Despite its importance, there is a great deal still unknown about soil OM chemical ecology. The development of sophisticated analytical methods have reshaped our understanding of soil OM composition, which is now believed to be comprised of plant and microbial products at various stages of decomposition. The methods also have recently been applied to study environmental change in various settings and have provided unique insight with respect to soil OM chemical ecology. The goal of this review is to highlight the methods used to characterize soil OM structure, source, and degradation that have enabled precise observations of OM and associated ecological shifts. Although the chemistry of soil OM is important in its overall fate in ecosystems, the studies conducted to date suggest that ecological function is not defined by soil OM chemistry alone. The longstanding questions regarding soil OM stability and recalcitrance will likely be answered when several molecular methods are used in tandem to closely examine structure, source, age, degradation stage, and interactions of specific OM components in soil.

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Introduction

Soil organic matter (OM) contains two-thirds of the terrestrial carbon storage in the world and more than twice as much carbon as the atmosphere (Schlesinger, 1991; Batjes, 1996). Soil OM is vital for microbial, plant, and animal life because it retains essential nutrients and water, and is critical for maintaining soil fertility and long-term agricultural sustainability (Howard and Howard, 1993; Kirschbaum, 1995; Baldock and Skjemstad, 2000; Lal, 2004; Leifeld and Fuhrer, 2005; Davidson and Janssens, 2006; Kramer and Gleixner, 2008; Leinweber et al., 2008). Soil OM acts as both a source and a sink of atmospheric CO_2, and plays an important role in the regulation of global climate change (Schlesinger, 1991; Batjes, 1996; Trumbore and Czimczik, 2008). Soil OM also interacts strongly with environmental pollutants (Simpson and Johnson, 2006; Chefetz and Xing, 2009), and thus, any shift in its structural composition may also impact the extent to which pollutants are sequestered in soils. Despite all of these critical functions, a large amount of soil OM, as well as other components of naturally occurring OM, are “molecularly uncharacterized” (Hedges et al., 2000). With the aid of analytical advancements, such as nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS), the scientific community is gaining a wealth of knowledge about soil OM composition (Hatcher et al., 2001; Simpson et al., 2011). More recently, this has been applied to better understand the chemical ecology of OM in terrestrial environments (Feng et al., 2008, 2010; Pautler et al., 2010; Clemente et al., 2011).

The current view of non-living soil OM (i.e., humic substances) is that it is comprised of a complex collection of plant and microbial products at various stages of abiotic and biotic decomposition (Sutton and Sposito, 2005; Kelleher and Simpson, 2006; Schmidt et al., 2011). Soil OM is chemically heterogeneous (Kögel-Knabner, 2002) and is considered to be the most complex natural mixture on earth, which confounds the study of its structure and reactivity in the environment (Simpson et al., 2011). Furthermore, the molecular-level role of soil OM in ecological responses to global climate change has not been studied in detail but is an emerging aspect of soil OM research. For example, understanding the fate of soil OM with ecological change will improve predictions of potential ecosystem shifts. The focus of this review is methods that assess soil OM structure, source, and turnover at the molecular level with emphasis on recent studies of environmental and ecological changes (Fig. 1). It should be noted that the topics covered here also are reviewed elsewhere, and in some cases in more detail, and readers are referred to these references for additional information (Amelung et al., 2009; Feng and Simpson 2011; Simpson et al., 2011). The overall purpose here is to underscore how recent molecular-level studies of soil OM have provided key information about soil OM molecular constituents and their chemical ecology on a larger scale. As illustrated in Fig. 1, molecular-level indices of soil OM can be linked to macroscopic- and ecosystem-level responses with respect to various aspects of ecological change. Commonly used molecular methods for soil OM constituents are summarized in Table 1. These methods have various strengths and are complementary in terms of their ability to delineate soil OM structures, sources, and stage of decomposition.

Methods for Elucidating Structure, Source and Degradation Stage

Nuclear Magnetic Resonance (NMR) Spectroscopy

Solid-state ^{13}C NMR methods are widely used to characterize soil OM because this method provides basic structural information of the whole soil sample with little or no pretreatment (Kögel-Knabner, 2000; Simpson et al., 2011). However, because only 1.1 % of carbon is in the form of ^{13}C (Simpson and Simpson, 2009), concentrating the OM prior to NMR analysis results in better quality spectra. It is important to note that the signal-to-noise ratio is inversely proportional to the square root of time. Therefore, longer experimental acquisition times do not necessarily result in improved spectral quality. The most common method of pretreatment is with hydrofluoric acid (HF), which dissolves minerals and subsequently concentrates OM such that suitable signal-to-noise can be achieved (Schmidt et al., 1997; Gonçalves et al., 2003). In addition, demineralization of soil samples prior to solid-state ^{13}C NMR analysis removes paramagnetic minerals that may interfere with the analysis. Rumpel and co-workers (2006) carefully evaluated soil OM alteration with HF treatment and concluded that there were no structural changes to OM that were detectable by solid-state ^{13}C NMR. However, they did note that complementary chemical methods (stable isotope and lignin phenol analysis) did detect the loss of carbohydrates and minor alteration to lignin with HF treatment (Rumpel et al., 2006). The benefits of using HF to pre-treat samples outweigh small losses in soil organic carbon because the resulting NMR spectra are better resolved and usually contain less noise, which is important for integration and semi-quantitative analysis of soil OM structure.

The most commonly applied solid-state ^{13}C NMR method is cross polarization magic angle spinning (CP-MAS) because it is faster and requires less instrument time than direct polarization methods (Simpson and Simpson, 2009). In the CP-MAS ^{13}C NMR experiment, magnetization is transferred from protons to nearby carbons (Pines et al., 1972), so data collection relies on the relaxation time of protons, which is considerably shorter than that of carbons (Simpson and Hatcher, 2004). Subsequent pulses can be performed faster than in direct polarization MAS of ^{13}C nuclei because carbon relaxation is substantially longer. Furthermore, the CP experiment makes use of protons that are usually abundant in OM, so in some cases sensitivity is enhanced. The main disadvantage of CP-MAS ^{13}C NMR is that it is not fully quantitative in an absolute sense, and it depends on the parameters used for acquisition, so it may underestimate aromatic and carbonyl

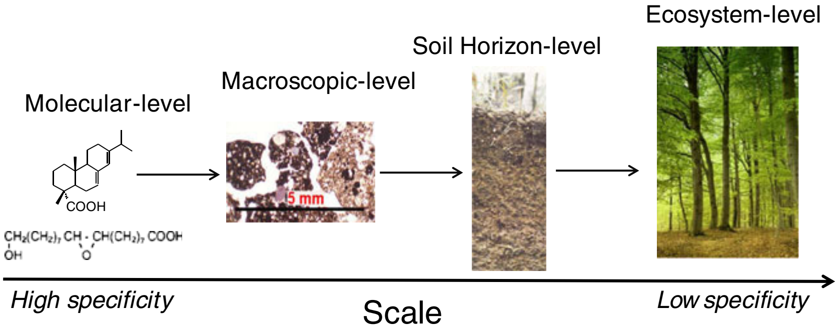


Fig. 1 Illustration of the relationships between different levels of measuring soil organic matter function. Molecular-level studies provide direct measures that can be used to understand soil organic matter responses at larger scales

Table 1 Overview and comparison of methods used to study soil organic matter molecular constituents

Soil organic matter structural component	Solid-state ^{13}C NMR	Solution-state ^{1}H NMR	Biomarker methods (GC-MS or LC-MS)
Black carbon/ biochar	Aromatic region (120–150 ppm) – potential overlap from lignin and other aromatics (Simpson and Hatcher, 2004).	May be detected provided that the components are soluble/extractable and contain sufficient protons for ^{1}H NMR detection.	Levoglucosan (solvent extractable; (Simoneit et al., 1999; Simoneit, 2002) Benzene polycarboxylic acids (BPCAs; Glaser et al., 1998; Haumaier, 2010)
Carbohydrates/sugars	*O*-alkyl region (50–90) and anomeric C signal (90–110 ppm) – potential overlap from amine C (~45 ppm) and methoxy C (Preston et al., 1997; Salloum et al., 2002).	May be detected but may overlap with resonances from amino acids (1-D ^{1}H spectra; Simpson and Simpson, 2009; Simpson and McKelvie, 2009). May also be identified using 2-D methods which provide better spectral resolution for chemical shift assignments of complex mixtures (Simpson, 2001; Simpson et al., 2001a, 2002b).	Free simple sugars can be extracted by organic solvents (Otto and Simpson, 2007). Acid hydrolysis can also be used to break up carbohydrates into simple sugars (Otto and Simpson, 2007).
Cutin and suberin	Polymethylene C resonances for cutin/ suberin (30–36 ppm; Deshmukh et al., 2005; Hu et al., 2000) and phenolic C (150–160 ppm; for suberin; Yan and Stark, 2000). Other long-chain and branched lipids can also resonate within the alkyl region (Preston et al., 1997; Salloum et al., 2002).	Terminal CH_3, mid-chain and branched CH_2 can be resolved in 1-D ^{1}H NMR spectra (Simpson et al., 2002b, 2003; Deshmukh et al., 2005; Feng et al., 2005, 2008; Clemente et al., 2011, 2012). Aromatic region is also more resolved and may be able to distinguish between lignin and suberin resonances using 2-D methods (Simpson et al., 2004; Kelleher and Simpson, 2006).	Cutin and suberin base hydrolysis products (refer to Table 2 for compounds; Otto et al., 2005; Otto and Simpson, 2006b; 2007; Feng et al., 2008)
Lignin	Aromatic/phenolic compounds as well as methoxy signal (Preston et al., 1997; Simpson and Hatcher, 2004). But these signals also arise from other soil organic matter compounds (methoxy in peptides and phenols in suberin) so lignin cannot be definitively identified or measured using solid-state ^{13}C NMR alone.	Lignin aromatic components can be identified (Simpson et al., 2004). Methoxy linkages can be determined using 2-D methods (namely ^{1}H-^{13}C experiments) that elucidate bonding and connectivity patterns (Simpson et al., 2004).	Lignin-derived phenols (monomers and dimers) using copper (II) oxidation (Hedges and Mann, 1979; Goñi et al., 1993; Otto and Simpson, 2006a; Clemente et al., 2011). Free phenols in solvent extracts can also be from suberin (i.e., ferulic acid) and are not necessarily lignin-derived (Otto et al., 2005; Otto and Simpson, 2007).
Microbial-derived compounds	Cannot be assigned solely from solid-state ^{13}C NMR due to overlap in alkyl and *O*-alkyl regions (see Fig. 2; Clemente et al., 2012).	Microbial-derived peptide signature (Simpson et al., 2007a; Clemente et al., 2012) and peptidoglycan signature (Pautler et al., 2010; Clemente et al., 2011, 2012).	Amino sugars (Amelung et al., 1996; Glaser et al., 2004) Bacteriohopanepolyols (BHPs; Talbot et al., 2001, 2003; Xu et al., 2009). Branched glycerol dialkyl glycerol tetraethers (GDGTs; Weijers et al., 2006b, 2007). Ergosterol (fungi; West et al., 1987) Hopanoids (Innes et al., 1997; Winkler et al., 2001; Shunthirasingham and Simpson, 2006). Phospholipid fatty acids (PLFAs; Frostegård and Bååth, 1996; Volkman et al., 1998; Zelles, 1999).
Peptides	Cannot be assigned solely from solid-state ^{13}C NMR due to overlap with resonances from other components (see Fig. 2; Clemente et al., 2012).	Peptide residues can be distinguished by other components and confirmed by comparing the intensity of the CH_2 peak to the CH_3 peak (if these signals are similar, then peptides are contributing more so than long chain aliphatic components (Feng et al., 2005, 2008, 2011a; Clemente et al., 2011; 2012).	Free amino acids can be extracted in organic solvents (Otto et al., 2005; Otto and Simpson, 2007). Amino acids can also be measured after acid hydrolysis (Otto et al., 2005; Otto and Simpson, 2007).

OM constituents (Mao et al., 2000; Hatcher et al., 2001). However, when all spectra are acquired using the same NMR parameters (namely CP contact time and relaxation time), relative quantification or semi-quantitative comparisons between samples of soil OM are possible (Preston et al., 1997).

The typical structural information that can be obtained from a CP-MAS ^{13}C NMR spectrum is illustrated in Fig. 2. This includes information about alkyl (0–50 ppm), *O*-alkyl (50–110 ppm), aromatic, and phenolic components (110–165 ppm) and carboxylic and carbonyl components (165–220 ppm; Malcolm, 1989; Preston et al., 1997; Baldock and Skjemstad, 2000; Salloum et al., 2002; Simpson et al., 2008). The heterogeneity of soil OM and strong dipolar coupling in the solid-state results in a number of broad resonances that can be derived from a variety of soil OM structural components. In most cases, however, solid-state ^{13}C NMR cannot be used to determine the precise structure of OM because signal overlap prevents definitive structural assignments. For example, several OM components contain aromatic rings. Signals within the aromatic region (110–

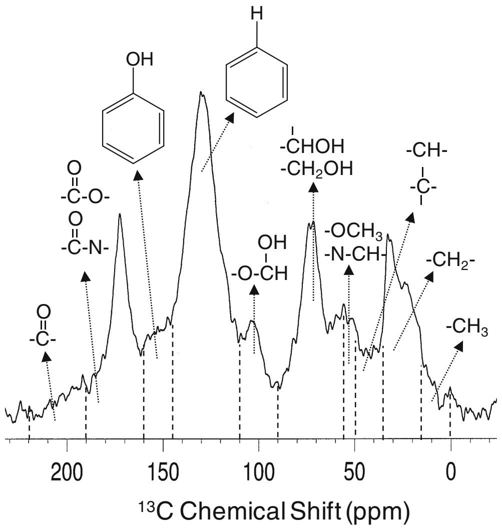


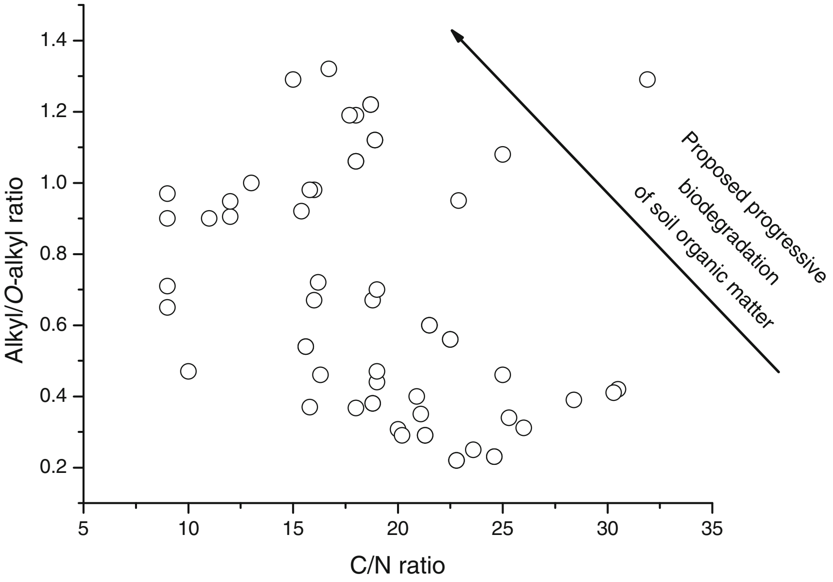
Fig. 2 A solid-state ^{13}C Cross Polarization Magic Angle Spinning (CP-MAS) Nuclear Magnetic Resonance (NMR) spectrum of a grassland soil (treated with hydrofluoric acid to concentrate the organic matter and remove paramagnetic minerals). Typical structural assignments for chemical shift regions are shown based on commonly used and accepted literature assignments (Malcolm, 1989; Preston et al., 1997; Baldock and Skjemstad, 2000; Salloum et al., 2002; Simpson et al., 2008)

165 ppm) may correspond to aromatic amino acids found in peptides/proteins, such as phenylalanine and tyrosine, lignin, black carbon, suberin, and/or tannins. Similarly, the alkyl carbon (0–50 ppm) and *O*-alkyl carbon (50–110) regions also correspond to a variety of components that can be assigned to a number of different OM sources, such as short- and long-chain lipids, plant waxes, amino acids in peptides/proteins, carbohydrates, and lignin (Malcolm, 1989; Preston et al., 1997; Baldock and Skjemstad, 2000; Salloum et al., 2002; Simpson et al., 2008).

The majority of the signals within the *O*-alkyl region correspond to labile and easily degraded OM constituents, such as carbohydrates and peptides/proteins as well as methoxy carbon that is found in both lignin and peptides (Malcolm, 1989). More recalcitrant and resistant forms of OM resonate within the alkyl region. For example, polymethylene carbon (30–34 ppm; Hu et al., 2000; Deshmukh et al., 2005) binds strongly to soil minerals (Feng et al., 2005) and is hypothesized to be resistant to biodegradation (Feng et al., 2008). A ratio of alkyl/*O*-alkyl carbon is often used to compare the relative stage of degradation of soil OM because the alkyl/*O*-alkyl ratio typically increases with progressive biodegradation of labile OM components (i.e., decline in *O*-alkyl compounds; Baldock and Preston, 1995; Simpson et al., 2008; Clemente et al., 2012).

The alkyl/*O*-alkyl ratio derived from solid-state ^{13}C NMR data may be analogous to the C/N ratio used to assess progressive biodegradation, but this has not been tested thoroughly. The C/N ratio of soil OM decreases with progressive biodegradation because plant-derived OM contains less nitrogen than microbial-derived OM (Wise and Schaefer, 1994; Szumigalski and Bayley, 1996; Almendros et al., 2000; Baumann et al., 2009). Thus, as plant material is degraded, the amount of carbon declines and the amount of nitrogen increases, which collectively results in an overall decline in the C/N ratio. Comparatively fresh OM, such as that found in plant residues, organic horizons, and light density fractions, typically has a higher C/N ratio as compared to whole soil OM (Otto and Simpson, 2005, 2006a; Sollins et al., 2009; Clemente et al., 2011). A comparison between compiled published ratios for a variety of soil types (Fig. 3) shows that there is a weak, inverse correlation between the C/N and alkyl/*O*-alkyl ratios for soil samples from a variety of ecosystems (Temperate, Arctic, and Antarctic). As the C/N ratio decreases with progressive OM

Fig. 3 Comparison of literature C/N vs. alkyl/*O*-alkyl ratios derived from solid-state ^{13}C NMR spectra. Data take from: (Beyer et al., 1995, 2001; Dai et al., 2001, 2002; Sjögersten et al., 2003; Simpson et al., 2008)



degradation, the alkyl/*O*-alkyl ratio increases because labile (*O*-alkyl) components are preferentially degraded compared to more resistant alkyl components. However, the large variation within the data points suggests that the relationship is not necessarily robust. NMR data collected using various solid-state ^{13}C parameters and field strengths may have resulted in variation of spectral quality as well as inconsistent errors in integration of NMR spectra. Further investigation is warranted and should focus on NMR data collected in an identical manner (i.e., from the same laboratory). Nonetheless, this compilation of literature data does show some consistency between the traditionally measured C/N and the more recently applied alkyl/*O*-alkyl ratio, with the latter providing more information about the depletion of labile constituents of OM and enrichment of alkyl OM constituents.

Solution-state NMR methods have been used more recently to study the composition of OM (Kingery et al., 2000; Simpson, 2001, 2002; Simpson et al., 2001a,b, 2002a,b, 2003, 2004, 2006, 2007a,b, 2011; Kelleher and Simpson, 2006; Kelleher et al., 2006; Feng et al., 2008, 2010, 2011a,b; Song et al., 2008; Simpson and Simpson, 2009; Pautler et al., 2010; Clemente et al., 2011, 2012). This method has several advantages over the more conventionally used solid-state ^{13}C NMR (Simpson et al., 2011; Clemente et al., 2012). The main advantage stems from the sensitivity obtained when using ^{1}H NMR experiments because of the abundance of hydrogen in soil OM, as well as improved resolution. Furthermore, multi-dimensional NMR methods can be employed that are used to confirm structural assignments (Fig. 4; Simpson et al., 2011). This provides an additional level of certainty in assignments of OM over that of solid-state ^{13}C NMR, in which overlap of components can prevent the accurate source apportionment of soil OM (Fig. 2). Additionally, diffusion editing experiments can be readily applied in solution-state NMR, and have been used to study large rigid components vs. small molecular components in OM, which also assists in monitoring soil OM compositional changes (Clemente et al., 2011, 2012; Feng et al., 2011a).

Solution-state ^{1}H NMR and two-dimensional solution-state NMR spectra from a forest soil extract are shown in Fig. 4. When used in tandem, these methods can identify specific components of OM, as illustrated. For example, distinct microbial-derived and lignin-derived components can be resolved using solution-state NMR techniques and assigned accurately, which is especially advantageous for recent studies that monitor environmental impacts on soil OM. Collectively, these studies have found that solution-state ^{1}H NMR is able to better identify variations in OM than solid-state ^{13}C NMR (Feng et al., 2008, 2010, 2011a; Pautler et al., 2010; Clemente et al., 2011, 2012). This likely is due to the sensitivity afforded by ^{1}H NMR vs. that of ^{13}C NMR techniques. Furthermore, as discussed previously, lignin and microbial-derived components (proteins) cannot be definitively assigned from solid-state ^{13}C NMR spectra alone due to the overlapping resonances typically observed (Table 1; Fig. 2). The main limitation of solution-state NMR methods is that only soluble OM components can be analyzed. Depending on the sample, only 52–79 % of the total carbon in a sample might be soluble (Simpson et al., 2007a). However, a recent comparison between solid-state ^{13}C and solution-state ^{1}H NMR data collected for three soils and soil fractions found that there was good consistency between the integration values for the major structural groups found in OM (Clemente et al., 2012). This suggests that solution-state ^{1}H NMR analysis of the soluble fraction is indeed representative of the total OM and provides an added level of specificity that cannot be obtained from solid-state ^{13}C NMR data alone.

Biomarker Methods

Biomarker methods have vastly improved the fundamental understanding of OM composition and ecological responses to environmental change (Feng et al., 2008, 2010, 2011c; Crow et al., 2009; Clemente et al., 2011). Biomarkers, which are analogous to OM fingerprints, are compounds that can be traced to a specific plant, microbial, or anthropogenic source because they retain their carbon backbones during abiotic and biotic degradation (Amelung et al., 2009). Biomarkers are used widely in marine organic geochemistry, but have also been used in soil OM studies to determine terrestrial OM sources as well as relative stages of decomposition (Hedges and Mann, 1979; Hedges et al., 1988; Van Bergen et al., 1997; Bull et al., 2000; Otto et al., 2005, 2006; Otto and Simpson, 2005, 2006a,b, 2007; Feng and Simpson, 2008).

Table 2 lists biomarkers that have been used in soil OM studies along with their commonly accepted sources. Many biomarkers are the products of chemolysis reactions on larger starting materials. For example, phospholipid fatty acids (PLFAs) and lignin-derived phenols and base hydrolysis products (a variety of hydroxyalkanoic acids) are parts of larger constituents found in soil OM. Lignin phenol monomers and dimers are isolated after CuO oxidation, which targets ether bonds in polymeric lignin (Hedges and Mann, 1979; Goñi et al., 1993; Otto and Simpson, 2006a). Base hydrolysis is used to break ester bonds found in cutin and suberin biopolymers, releasing components that are amenable to analysis (Otto and Simpson, 2006b; Mendez-Millan et al., 2010).

Biomarkers are predominantly measured using gas chromatography – mass spectrometry (GC-MS), a method that facilitates separation and accurate quantification of individual OM components (Simoneit 2005; Amelung et al., 2009). In most cases, biomarker compounds must be derivatized to

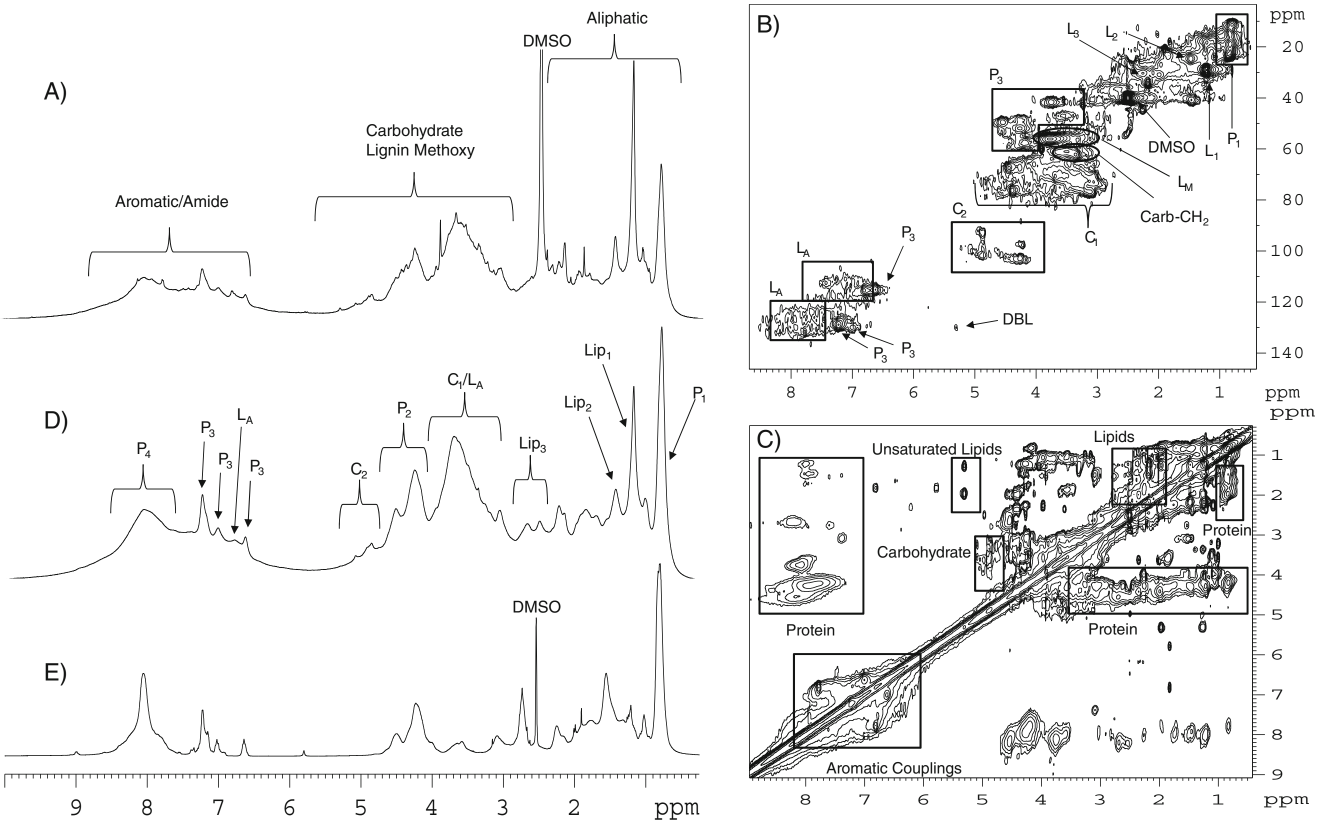


Fig. 4 Combining one-dimensional (1-D) and two-dimensional (2-D) nuclear magnetic resonance (NMR) to extract structural information about sodium hydroxide extractable soil organic matter isolated from a grassland-forest transition A horizon (Dark Grey Chernozem from Alberta, Canada; Kelleher and Simpson, 2006; Simpson et al., 2007a). **A)** Simple ^{1}H 1-D NMR which can be used to make generic assignments. **B)** ^{1}H-^{13}C Correlation Spectroscopy (Heteronuclear Multiple Quantum Coherence; HMQC) which reduces the spectral overlap *via* dispersion into two dimensions and also provides information about ^{1}H-^{13}C connectivity. A range of assignments are possible and include: L_A=Lignin Aromatics; L_M=Lignin Methoxy; C_1=carbohydrate; C_2=anomeric units in carbohydrates; Carb-CH_2=CH_2 in carbohydrates; L_1 polymethylene chains (mainly lipids and waxes); L_2= units β to ester, acid or double bond; L_3=units α to carboxyl acid or carbonyl of an ester; DBL=double bond from lipids; P_1=methyl rich amino acid side chains (such as valine) in proteins/peptides; P_2=alpha units in proteins/peptides; and P_3=aromatic amino acids. DMSO – dimethyl sulphoxide (solvent). **C)** ^{1}H-^{1}H Total Correlation Spectroscopy (TOCSY) helps determine how the various units are connected and also assists with confirming the assignments from ^{1}H-^{13}C Correlation Spectroscopy (B). For simplicity TOCSY assignments are given in terms of the major components present. Once structures are identified by 2-D NMR, assignments can be transferred back to the 1-D spectrum. **D)** A diffusion edited ^{1}H NMR spectrum with assigned resonances. Diffusion editing highlights the macromolecular components and small molecules such as metabolites and the DMSO solvent are removed by diffusion editing. Once complete, assignments can be further confirmed by comparison to standards. **E)** Spectrum of Bovine Serum Albumin (commonly used protein standard) to represent a general protein structure. A comparison of **D)** and **E)** helps identify the protein profile in soil organic matter extracts. The protein has been shown to mainly arise from soil microbes that are ruptured during the alkaline extraction process (Simpson et al., 2007a)

make them more volatile prior to GC analysis (Otto and Simpson, 2006a,b, 2007; Mendez-Millan et al., 2010). In some cases, larger intact biomarkers such as bacteriohopanepolyols (BHPs; Talbot et al., 2001, 2003, 2007a,b; Xu et al., 2009) and branched glycerol dialkyl glycerol tetraethers (GDGTs; Weijers et al., 2006a, 2011; Peterse et al., 2010; Zhu et al., 2011) are measured using liquid chromatography (LC) – MS. With LC-MS, the side chain information for isolated BHPs provides an additional level of organism specificity, sometimes to the species level (Fox et al., 1998; Talbot et al., 2008; Xu et al., 2009). In contrast, analysis of the cleaved hopanoid by GC-MS is carried out after removal of the side chain (Innes et al., 1997; Winkler et al., 2001; Shunthirasingham and Simpson, 2006).

In addition to providing quantitative source information, biomarkers also can be used to study OM degradation and turnover (Table 3). OM can undergo a variety of processes in soil, which include abiotic and biotic degradation as well as preservation of intact constituents (Fig. 5; Otto and Simpson, 2005). For example, plant steroid biodegradation can vary with respect to soil type and environmental conditions including moisture and temperature. Common plant steroids include β-sitosterol, stigmasterol, and stigmastanol (Otto and Simpson, 2005). Once in soil, these steroids can

Table 2 Commonly observed biomarkers in soil organic matter and their potential source(s). Compiled from: (Holloway and Deas, 1973; Tulloch, 1976; Weete, 1976; Hedges and Mann, 1979; Baker, 1982; Holloway, 1982; Harwood and Russell, 1984; Hedges et al., 1988; Kolattukudy and Espelie, 1989; Goñi et al., 1993; Bianchi, 1995; Amelung et al., 1996, 2009; Frostegård and Bååth, 1996; Innes et al., 1997; Van Bergen et al., 1997; Volkman et al., 1998; Zelles, 1999; Bull et al., 2000; Winkler et al., 2001; Glaser et al., 2004; Hopmans et al., 2004; Otto and Simpson, 2005, 2006a,b, 2007; Otto et al., 2005, 2006; Shunthirasingham and Simpson, 2006; Weijers et al., 2006b; Feng and Simpson, 2007, 2008; Feng et al., 2008, 2010; Simpson et al., 2008; Xu et al., 2009)

Biomarker Compound(s)	Potential Source(s)
C_{21}–C_{35} (odd numbered) n-alkanes	Plants (waxes), fungi
C_{16}–C_{34} (even numbered) n-alkanols	Plants (waxes and suberin)
C_{12}–C_{24} (even numbered) n-alkanoic acids	Pants (waxes,cutin and suberin), fungi
C_{16} and C_{18} iso-alkanoic acids	Bacteria and fungi
Glucose, mannose, sucrose	All organisms
Trehalose	Fungi, insects, some plants
Levoglucosan	Cellulose (Biomass burning)
Amino sugars (glucosamine, galactosamine, mannosamine, muramic acid[a]) and Phospholipid fatty acids (PLFAs)[b]	Bacteria and fungi
Hopanoids, Bacteriohopanepolyols (BHPs) and branched glycerol dialkyl glycerol tetraethers (GDGTs)	Bacteria
Ergosterol	Fungi
Cholesterol	All organisms
β-sitosterol, stigmasterol, stigmastanol, stigmastan-3-one, stigmasta-3,5-dien-7-one, sitosterone	Plants (steroids)
Diterpenoids	Conifers
Triterpenoids	Angiosperms
Vanillin, vanillic acid, acetovanillone, syringaldehyde, syringic acid, acetosyringone, *p*-coumaric acid, ferulic acid[c]	Lignin (angiosperms and gymnosperms)
C_{16} and C_{18} ω-hydroxyalkanoic acids[d]	Cutin and suberin
C_{20}–C_{30} (even numbered) α,ω-alkanedioic acids[d]	Suberin and plants (waxes)
C_{16} and C_{18} α,ω-alkanedioic acids[d]	Cutin
C_{16} and C_{18} di- and trihydroxyalkanoic acids[d]	Cutin and suberin
C_{16} and C_{22}–C_{26} (even numbered) α-hydroxyalkanoic acids)[d]	Plants and fungi

[a] muramic acid is a biomarker for bacteria (Glaser et al., 2004)

[b] 18:2ω6,9*c* PLFA is specific to fungi; 16:1ω7*c*, *cy*17:0, 18:1ω7*c* and *cy*19:0 PLFAs are specific to Gram-negative bacteria; and *i*14:0, *i*15:0, *a*15:0, *i*16:0, *i*17:0, and *a*17:0 PLFAs are specific to Gram-positive bacteria (Feng and Simpson, 2009)

[c] ferulic acid can also be an indicator of suberin. The exact source is dependent on the extraction method used (solvent extraction vs. CuO oxidation; (Otto et al., 2005; Otto and Simpson, 2007))

[d] α-hydroxyalkanoic acids are isolated using base hydrolysis (Otto and Simpson, 2007)

be preserved or biodegraded (Fig. 5). The ratio of the precursor biomarkers to the degradation products (Table 3) can be used to assess plant steroid fate in soil OM (Otto and Simpson, 2005). Similarly, other biomarker ratios can be used to study a variety of processes related to OM dynamics, such as the fate of plant- or microbially-derived compounds in soil and the impact of forest fires on soil OM composition (Table 3). Acid-to-aldehyde ratios for lignin-derived phenols commonly are applied to study the stability of lignin in various ecosystems (Hedges et al., 1988; Goñi et al., 1993; Otto and Simpson, 2006a; Feng et al., 2008; Crow et al., 2009; Thevenot et al., 2010; Clemente et al., 2011). For example, the acid-to-aldehyde ratio of vanillyl- and syringyl-type phenols increases with progressive lignin oxidation, and thus, is a useful measure of lignin degradation in soil (Table 3; Goñi et al., 1993; Otto and Simpson, 2006a). Other ratios relating to cutin and suberin also provide information regarding the relative degradation state of these resistant components of soil OM (Table 3; Otto and Simpson, 2006b). PLFA ratios also can be used to quantify microbial stress due to declining substrate availability (Table 3; Feng and Simpson, 2009; Pautler et al., 2010).

Biomarker methods have several advantages over NMR techniques (Table 1). MS is generally more sensitive than NMR and is able to detect low concentrations of biomarkers (nanogram range or lower depending on the instrument and compound of interest). Furthermore, separation *via* GC and LC enables the identification and quantification of individual components, whereas NMR spectra may have overlapping signals that prevent precise quantification of individual

Table 3 Soil organic matter biomarker ratios used to monitor ecological responses to environmental change

Biomarker	Degradation Ratios	Interpretation	Ecological Monitoring
Plant-derived steroids	Precursors (β-sitosterol+stigmasterol+stigmastanol)/Degradation products (stigmastan-3-one+stigmasta-3,5-dien-7-one+sitosterone)	Ratio of precursors/degradation products decreases with increasing soil organic matter degradation	Degradation vs. preservation of plant-derived components in soils from various environments (Otto and Simpson, 2005). Impact of forest fires on soil organic matter oxidation (Otto et al., 2006).
Conifer-derived diterpenoids	Precursors (abietic acid+dehydroabietic acid)/Degradation products (abieta-6,8,11,13-tetraenoic acid+abieta-8,11,13,15-tetraenoic acid)	Ratio of precursors/degradation products decreases with increasing soil organic matter degradation	Degradation vs. preservation of plant-derived components in soils from various environments (Otto and Simpson, 2005). Impact of forest fires on soil organic matter oxidation (Otto et al., 2006).
Cutin	ω-C_{16} hydroxy acids/ΣC_{16} hydroxy acids (ω-hydroxy C_{16} acid+α,ω-dioic C_{16} acid +ΣC_{16} mid-chain-substituted acids)	Ratio of ω-C_{16} hydroxy acids/ΣC_{16} hydroxy acids increases with progressive cutin degradation in soils	Degradation vs. preservation of plant-derived components in soils from various environments (Otto and Simpson, 2006b). Increased cutin sequestration with soil warming (Feng et al., 2008). Increased cutin sequestration with free atmospheric CO_2 enrichment and N fertilization (Feng et al., 2010) Investigation of compound-specific temperature sensitivity in a 1 year laboratory incubation (Feng and Simpson, 2008).
Lignin	Vanillic acid/vanillin Syringic acid/syringaldehyde	Both ratios increase with increasing lignin degradation.	Enhanced lignin oxidation with soil warming (Feng et al., 2008). Enhanced lignin oxidation with free atmospheric CO_2 enrichment and N fertilization (Feng et al., 2010). Enhanced lignin degradation and preservation in soil density fractions with linkages to environmental controls (Clemente et al., 2011). Investigation of compound-specific temperature sensitivity in a 1 year laboratory incubation (Feng and Simpson, 2008).
Phospholipid fatty acids (PLFAs)	Monoenoic PLFAs/saturated PLFAs	Ratio decreases with substrate limitations.	Used to assess soil organic matter availability with permafrost active layer detachments in the Canadian High Arctic (Pautler et al., 2010).
	Specific PLFA ratios: *cy*17:0/16:1ω7*c* and *cy*19:0/18:1ω7*c*	Ratios increase with substrate limitations.	Used to monitor microbial activity with simulated soil temperature increases (Feng and Simpson, 2009).

components. Biomarker methods also can be applied more routinely than NMR because the relatively economical and low maintenance GC-MS and LC-MS instruments are more widely available than more costly NMR spectrometers. Despite these advantages, biomarker methods only capture a small percentage of soil OM. For example, a sequential extraction method that released a large variety of biomarkers only accounted for 17 % of the total soil organic carbon in the sample (Otto and Simpson, 2007). This is in sharp contrast to the high percentage of OM that can be measured using solid-state ^{13}C NMR (practically all of the OM) and solution-state NMR (52–79 %). It is noteworthy that biomarker and NMR methods are complementary (Otto and Simpson, 2007; Simpson et al., 2008). For example, degradation ratios for plant-derived steroids, cutin, and suberin, and lignin-phenols corresponded to degradation trends measured using alkyl/*O*-alkyl ratios by solid-state ^{13}C NMR for four prairie soils (Simpson et al., 2008). Finally, biomarker methods are targeted and selective methods, and one would employ a specific extraction procedure for a specific group (e.g., CuO oxidation to isolate lignin phenols; Otto and Simpson, 2007). NMR methods are non-targeted, and because NMR is non-selective, no *a priori* knowledge about the sample is needed. Thus, depending on the information required, NMR or biomarkers can be used. However, the examples discussed later in this paper demonstrate the benefits of using both techniques in tandem.

Isotopic Methods

Soil OM turnover and relative age can be assessed by using biomarkers in combination with isotopic methods (Amelung et al., 2009; Feng et al., 2011c; Mendez-Millan et al., 2011) or radiocarbon techniques (Eglinton et al., 1996; Hou et al.,

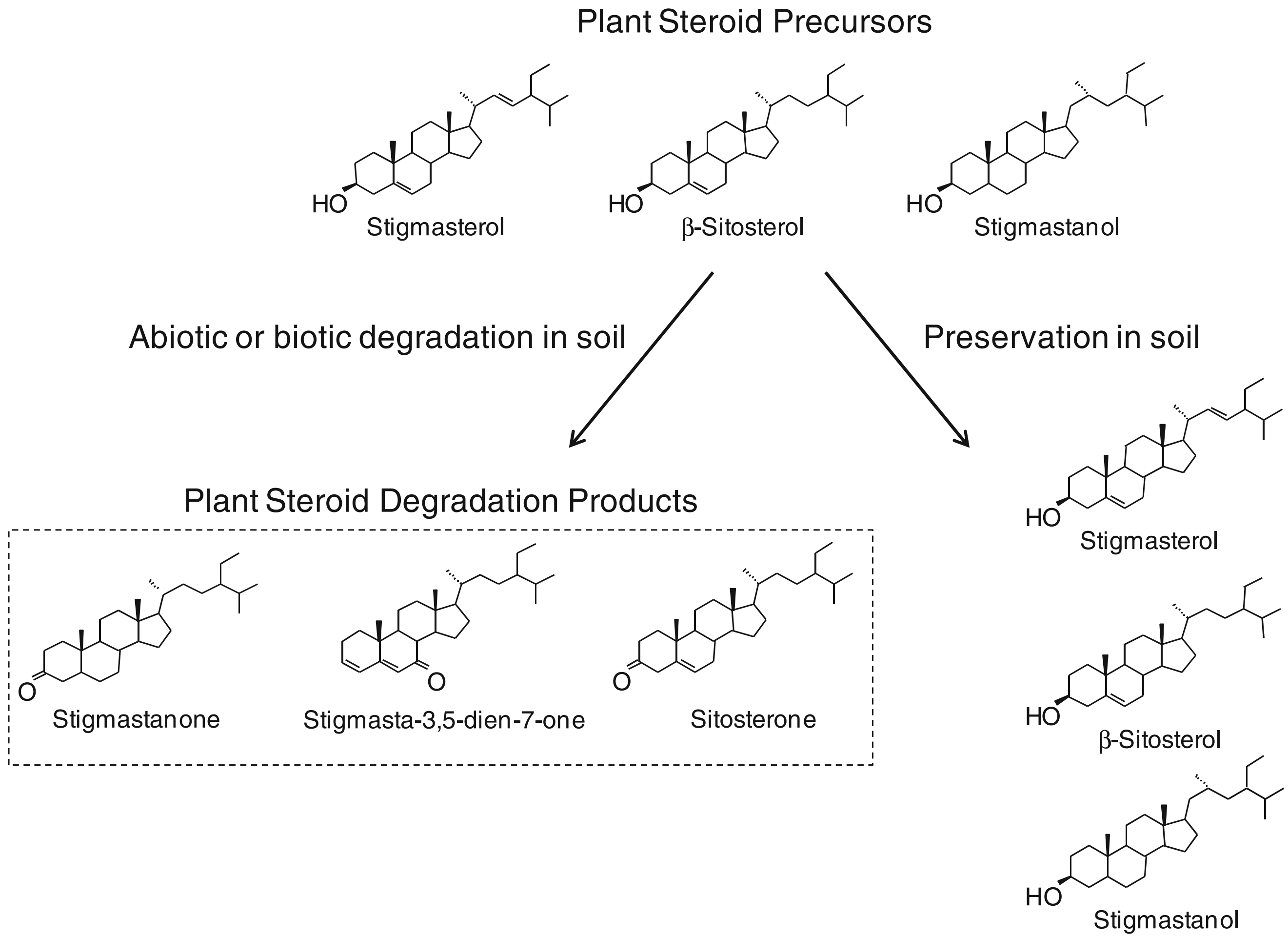


Fig. 5 Proposed routes of biomarker preservation and degradation in soil (Otto and Simpson, 2005). Plant steroids can be preserved intact (precursor compounds) or degraded. A ratio of precursor compounds vs. degradation products can be used to monitor various ecological processes (see Table 3 for more information)

2010; Ingalls et al., 2010). For example, compound specific isotope analysis (CSIA) has been used to measure the turnover and dynamics of SOM components in disturbed systems where new soil carbon inputs carry a distinct ^{13}C isotopic signature as compared to that of native (older) soil OM. Similar methods can be used when labelled compounds, such as when ^{13}C or ^{15}N enriched substrates, are introduced to the soil (Kuzyakov and Bol, 2005; Providoli et al., 2006; Huygens et al., 2008; Dijkstra, 2009; Dijkstra et al., 2011; Glaser et al., 2012; Hatton et al., 2012). The variation in ^{12}C vs. ^{13}C isotopes (expressed as $\delta^{13}C$) can also be used to identify variations in C3 vs. C4 plant-derived OM as well as to investigate OM turnover mechanisms (Blagodatskaya et al., 2011; Christensen et al., 2011; Dijkstra et al., 2008, 2010; Dümig et al., 2009; Mendez-Millan et al., 2012; Morgan et al., 2011; Torn et al., 2011). The use of radiocarbon to examine the fate of specific OM components in soil over decadal and millennial timescales can be examined by simultaneously using ($\Delta^{14}C$) to measure soil OM age and biomarkers to evaluate specific compounds. This technique, referred to as compound specific radiocarbon analysis (CSRA), was developed to study the fate of OM in marine systems (Eglinton et al., 1996), and has only been used occasionally to measure the radiocarbon age of specific soil OM components (biomarkers; Rethemeyer et al., 2005; Matsumoto et al., 2007; Kramer et al., 2010). It should be noted that radiocarbon methods have been used to examine whole soils and soil density-size fractions in a number of studies that have defined the potential for using these methods in studying soil OM chemical ecology (Rumpel et al., 1998; Froberg et al., 2003; Rethemeyer et al., 2004, 2005; Czimczik et al., 2005; Trumbore, 2006, 2009; Carbone et al., 2007; Czimczik and Trumbore, 2007). In the future, application of CSRA will develop our understanding of the difference between total soil OM residence time and that of specific OM components.

NMR and Biomarker Studies of Ecological Responses to Environmental Change

Soil Organic Matter Structural Shifts with Soil Warming

Increasing atmospheric temperatures are likely to impact the rate of soil processes. Warming is also likely to alter SOM composition *via* changes in vegetation and microbial diversity, which may subsequently shift the decomposition patterns of SOM (Biasi et al., 2005; Frey et al., 2008). Microbial degradation of labile soil OM also may be accelerated (Davidson and Janssens, 2006) so that microbial diversity as well as the quality of soil OM stocks may change. The manner in which soil OM chemistry may shift is of critical importance to the global carbon cycle but these process have not been carefully investigated using molecular-level OM characterization techniques (Davidson and Janssens, 2006; Trumbore and Czimczik, 2008). Traditional soil OM degradation studies often examine the amount of CO_2 respired, which is also used to calculate soil OM temperature sensitivity (Q_{10}). Attempts have been made to relate these measures to soil OM quality. For example, Fang et al. (2005) did not observe any significant difference in CO_2 respired during soil incubation at different temperatures (expressed as Q_{10}) and suggested that the soil OM resistant pool is just as temperature sensitive as the more labile constituents. However, this study did not employ molecular-level measurements. Feng and Simpson (2008) monitored OM biomarkers along with CO_2 respiration in a one-year laboratory incubation and reported that different soil OM components exhibited varying temperature sensitivity (Q_{10}) values. They concluded that soil respiration measurements and Q_{10} values may not be reliable indicators of temperature responses for individual soil OM components. Long-term soil warming has shown that microbial respiration declines over time (Frey et al., 2008), and has also lead to the hypothesis that microbial activity diminishes after labile substrates are depleted–but what happens to the hypothesized “stable” components such as lignin, cutin and suberin?

Feng et al. (2008) employed both NMR and biomarker methods to study soil OM composition before and after 14 months of *in situ* warming (+5°C) in a temperate mixed forest to determine the fate of specific OM components. The results showed a significant change in soil OM composition with soil warming. Simple carbohydrates and lignin phenols declined with soil warming. Lignin-phenol acid-to- aldehyde ratios also significantly increased, which suggested that lignin was susceptible to advanced biodegradation brought on by soil warming. PLFA analysis also showed that the concentration of the fungal PLFA doubled, while bacterial PLFA concentrations did not change. Cutin-derived biomarkers increased with soil warming, with enrichment in the more recalcitrant OM fraction. These results were confirmed by examining the composition of humic substances by using solution-state NMR. This study confirmed that lignin-derived soil OM, which is often hypothesized to be stable (Knorr et al., 2005), is in fact sensitive to accelerated decomposition by native soil microbes at elevated temperatures. These results demonstrate a significant shift in soil OM composition and substrate use associated with the enhanced microbial activity brought on with soil warming. Additional studies are needed to delineate whether or not perceived stable components, namely lignin- and cutin-derived soil OM, are stable in the long-term. The results of these studies also should be further tested using soils from other ecological settings to examine the role of environmental factors on the chemical ecology of soil OM.

In addition to studies that focus on carbon storage in temperate regions, there is growing concern about carbon stocks in Arctic regions. As much as 50 % of the global soil carbon is stored below ground in the Arctic (Tarnocai et al., 2009). Much of this carbon has been stored in permanently frozen soils (i.e., permafrost) and is characterized by the accumulation of labile constituents in soil OM. However, increased temperatures in northern regions could result in enhanced soil OM degradation and subsequent positive feedback to additional climate change *via* additional atmospheric CO_2 flux as the active layer of permafrost soils increases (Beer, 2008). Several studies have used molecular-level methods to examine the nature of arctic soil OM and to determine if it is more susceptible to accelerated biodegradation than more commonly studied soil OM from temperate regions (Dai et al., 2001, 2002; Sjögersten et al., 2003; White et al., 2007). White et al. (2007) used pyrolysis GC-MS to examine 19 soils collected from a variety of circumpolar regions and found that these soils were carbohydrate rich. Solid-state ^{13}C NMR characterization studies of soil OM from the Arctic, Antarctic, and northern alpine regions have collectively reported that alkyl and *O*-alkyl structures dominated the NMR spectra (Beyer et al., 1995, 1997, 2001; Dai et al., 2001, 2002; Sjögersten et al., 2003; Pautler et al., 2010; Pedersen et al., 2011). These studies provide the underlying basis for concern since labile OM constituents (i.e., *O*-alkyl components) are particularly susceptible to climate change. A detailed study by Pautler et al. (2010) that used both NMR and biomarker techniques to examine the impact of permafrost melt on soils from Nunavut, Canada revealed that not only do previously disturbed sites have low *O*-alkyl carbon content but PLFA stress indicators showed that these labile substrates are depleted rapidly once released from permafrost. This study highlights the fact that soil OM in the High Arctic is indeed susceptible to accelerated decomposition with the onset of warming and subsequent permafrost thawing.

Soil Organic Matter Structural Shifts with Elevated Atmospheric CO_2 and Nitrogen Deposition

Increasing atmospheric CO_2 levels and nitrogen deposition also are predicted scenarios for environmental change that may impact soil OM chemical ecology (Lichter et al., 2008; Billings et al., 2010). NMR and biomarker methods in forest floor and surface soils from the Duke Forest free air CO_2 enrichment (FACE) experiment after 10 years of elevated CO_2 and 2 years of N fertilization were used to examine ecological shifts with respect to soil OM composition and turnover (Feng et al., 2010). Plant steroid concentrations in O horizons increased with elevated atmospheric CO_2 levels. Cutin-derived compounds in soil OM also increased, perhaps as a direct result of increased pine needle inputs into the soil with elevated atmospheric CO_2. Solution-state ^{1}H NMR also showed an enhancement of CH_2 resonances in soil extracts from the elevated atmospheric CO_2 samples. The same trend was observed with nitrogen fertilization, which suggested that soil OM degradation patterns shifted that resulted in an enrichment of more recalcitrant cutin-derived components. Enhanced nitrogen deposition also altered the microbial PLFA composition and the extent of lignin oxidation, which highlights the important relationship between soil OM composition and microbial activity and diversity. It is noteworthy that OC contents of the soils did not change with either FACE or N fertilization, thus demonstrating that soil OM compositional shifts may not be detected by elemental composition alone.

To further assess the fate of recalcitrant components in soil OM with FACE, CSIA of hydrolyzable aliphatic compounds (which mainly arise from cutin and suberin) was performed such that turnover times of these theoretically recalcitrant components could be ascertained (Feng et al., 2011c). Importantly, the CO_2 used in the FACE experiment was ^{13}C-depleted so that molecular tracer studies could be used to follow carbon biogeochemistry. The isotopic ratios revealed that these lipids have a mean residence time ranging from 32 to 34 years, and indicated that cutin and suberin may undergo relatively fast transformation to forms that are not extractable by using base hydrolysis methods. Many studies have showed that cutin and suberin compounds sorb strongly to clay mineral surfaces (Feng et al., 2005; Simpson et al., 2006; Simpson and Johnson, 2006), and this interaction may render them non-extractable. Molecular-studies may be limited by the extractability of soil OM components.

Summary and Future Directions

Molecular-level techniques have facilitated our ability to identify key findings with respect to soil OM composition and shifts with environmental change. The fate of soil OM and its chemical ecology is of utmost concern as we attempt to understand various aspects of global climate change. The molecular methods highlighted within this short review have demonstrated sensitivity for identifying chemical changes in soil OM, and these changes have been directly related to ecological shifts in soil OM dynamics. In some cases, these changes were not observed by classic methods of measuring soil characteristics (i.e., CO_2 respiration and soil carbon content).

Molecular methods are able to measure soil OM components directly, thus minimizing possible misinterpretation of the data. For instance, chemical methods enable the detection and measurement of soil OM components that have an established source (plant-, microbial- and/or anthropogenically-derived) and ecological function. Sophisticated NMR methods have shown that humic substances, which have been traditionally viewed as large, stable macromolecular components, are better described as a complex mixture of plant- and microbially-derived compounds at various stages of decomposition (Sutton and Sposito, 2005; Kelleher and Simpson, 2006; Schmidt et al., 2011).

Because molecular methods have been applied only to analysis of soil OM recently, there are probably additional undocumented compounds still to be discovered. There is a continuing need to identify further soil OM compounds, sources, and their ecological reactivities. Future research must focus on the identification of novel soil OM components by using both MS and NMR methods. Furthermore, the advancement of analytical technologies will likely improve the capabilities for studying complex mixtures along with improvements in detection levels and resolving power.

An important factor to consider when applying molecular-level methods is pre-treatment/extraction steps that may be required, and that may or may not be exhaustive for a specific group of OM compounds. For example, biomarker yields vary from soil to soil, so extractible biomarker measures may or may not be indicative of soil OM composition. The strong sorption of some soil OM components may result in difficulties with respect to biomarker methods. Clay minerals selectively preserve soil OM components, and thus, analyzing the absolute quantities of some of these components is challenging. For example, protected biomarkers are extractable after HF treatment used for demineralization (Mead and Goñi, 2008). Solution-state NMR analysis of the soil humin fraction, which is operationally defined as non-extractable, also is possible after demineralization and exhaustive extraction (Simpson et al., 2007b).

Standardization of extraction protocols would facilitate direct comparisons of results obtained from different laboratories, and also could identify the precise shortcomings of extraction methods. Clemente et al. (2011) concluded that in addition to soil minerals, soil OM components also may be responsible for the preservation of specific OM compounds, and proposed that OM-OM interactions may also relate to long-term persistence of OM in the environment. As such, one must always consider the mineral phase as well as protection from other OM components when assessing the ecological stability of compounds because compounds that are considered labile can be preserved through interactions with mineral and other OM components. Therefore, stability may not necessarily stem from OM chemistry alone but from its reactivity with both living and non-living components of soil.

Recent research using radiocarbon methods has provided additional support for the interplay between OM chemistry and physical protection. For example, Marschner and coworkers (2008) found that mineral-associated OM had the oldest radiocarbon ages when compared to whole soils and other density fractions. Similarly, O'Brien et al. (2012) found that mineral-stabilized OM had older radiocarbon dates than OM that was not mineral-bound and concluded that OM chemistry was not a good indicator of soil OM resistance to microbial decomposition and long-term stability in soil. Kleber et al. (2011) also reported that soil OM radiocarbon age did not correlate to structure or thermodynamic stability. Future soil OM research should combine molecular methods that identify OM constituents with radiocarbon methods to determine age. For example, CSRA of lignin-derived phenols would help ascertain the relative residence time of lignin-derived OM in soil environments.

In a recent review aptly entitled, "What is recalcitrant soil OM?", Kleber (2010) discusses the many factors that determine the overall fate of OM in soil. It is clear that to answer this question, we must go beyond studying soil OM chemistry alone. However, the advanced chemical methods described within this review have significantly changed our view on soil OM structure and ecological function. Future efforts that use these methods will undoubtedly help us answer long-standing and complex questions about soil OM recalcitrance. The use of a host of methods simultaneously will improve the quality of information and will provide answers to pressing questions that relate to soil OM chemistry, ecological stability, and reactivity. The overall goal of this research is to identify molecular level indicators that can be directly used to predict ecological responses (Fig. 1) to various aspects of environmental change. These indicators will in turn result in improved management and monitoring strategies for our changing world.

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REVIEW ARTICLE

Tracing Hidden Herbivores: Time-Resolved Non-Invasive Analysis of Belowground Volatiles by Proton-Transfer-Reaction Mass Spectrometry (PTR-MS)

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Abstract Root herbivores are notoriously difficult to study, as they feed hidden in the soil. However, root herbivores may be traced by analyzing specific volatile organic compounds (VOCs) that are produced by damaged roots. These VOCs not only support parasitoids in the localization of their host, but also may help scientists study belowground plant-herbivore interactions. Herbivore-induced VOCs are usually analyzed by gas-chromatography mass spectrometry (GC-MS), but with this off-line method, the gases of interest need to be preconcentrated, and destructive sampling is required to assess the level of damage to the roots. In contrast to this, proton-transfer-reaction mass spectrometry (PTR-MS) is a very sensitive on-line, non-invasive method. PTR-MS already has been successfully applied to analyze VOCs produced by aboveground (infested) plant parts. In this review, we provide a brief overview of PTR-MS and illustrate how this technology can be applied to detect specific root-herbivore induced VOCs from *Brassica* plants. We also specify the advantages and disadvantages of PTR-MS analyses and new technological developments to overcome their limitations.

Keywords Chemical ecology · Root herbivory · Trace gas analysis · Induced indirect defense · Mass spectrometry · Volatile organic compound (VOC)

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Introduction

Belowground herbivores can cause substantial damage to plant roots, which in many cases has a more severe impact on plant fitness than shoot damage (Gerber et al., 2007; Johnson et al., 2007). Nevertheless, interactions between belowground herbivores and their hosts have been much less-studied than those of their aboveground counterparts. One of the reasons is the obscurity of root-herbivore interactions in the soil, which also means that root damage cannot be assessed as easily as shoot damage. Plants generally need to be sampled destructively to assess how much and where root feeding has occurred. To overcome this drawback and to non-invasively visualize the activities of root herbivores, techniques such as X-ray tomography and magnetic resonance imaging (MRI) have been successfully applied (Johnson et al., 2007; Jahnke et al., 2009). In addition, the development of new methods, for example, root area determination by using electrical potential measurements, may lead to novel approaches that help to monitor the feeding activities of root herbivores *in vivo* (Cao et al., 2010). Here, we present a novel approach to tracing the feeding activities of root herbivores that involves the detection of herbivore-induced volatile organic compounds (VOCs) as a method for damage assessment (van Tol et al., 2001; Rasmann et al., 2005; Kaplan et al., 2008).

Despite the paucity of data, it has become evident that responses induced below ground in many respects resemble those found in aboveground plant-herbivore interactions. Just as in shoots, responses induced by herbivores may be both local and systemic, either within the root system or the whole plant, and comprise a wide range of defense compounds, such as alkaloids, phenolics, cardiac glycosides, and glucosinolates (Kaplan et al., 2008; Rasmann et al., 2009; van

Dam, 2009; Hiltpold et al., 2011; Pierre et al., 2011a). In addition, root herbivory leads to the induction of volatile organic compounds (VOCs) that can be involved in indirect defenses below ground, by attracting the enemies of the attackers (van Tol et al., 2001; Rasmann et al., 2005; Pierre et al., 2011b). The role of VOCs might be even more important for belowground communities in the rhizosphere, as they serve herbivores and parasoitoids as cues for host localization in an environment where visual cues are lacking (Rasmann et al., 2005; van Dam, 2009). Indeed, recent studies have revealed various root-produced VOCs that play a role in plant-environment interactions. Maize roots attacked by larvae of the Western cornworm (*Diabrotica virgifera virgifera*) emit (*E*)-β-caryophyllene, a sesquiterpenoid that attracts entomopathogenic nematodes (Rasmann et al., 2005), while *Brassica* plants infested with the larvae of the cabbage root fly (*Delia radicum*) emit sulfides that attract ground-dwelling predatory beetles (Ferry et al., 2007) and also various other VOCs that may be important cues for parasitoids of these root herbivores (Neveu et al., 2002; Pierre et al., 2011b).

Many compound classes that have been identified to play a role in belowground plant-environment interactions also are known from aboveground organs. Despite the overlap in defense strategies and compounds, there are striking differences in the VOCs produced by roots and shoots. For example, green leaf volatiles (GLVs) are commonly emitted by aboveground tissues of almost all higher plants after damage (Hansson et al., 1999; Barth and Schmid, 2001). However, they are not emitted when plant roots are artificially damaged or infested by herbivores (Steeghs et al., 2004), although they can be detected in minute amounts when plant roots are ground up (Matthias Erb, pers. comm.). Furthermore, the emission of sulfides, which often decreases when *Brassica* plants are damaged by aboveground herbivores (Blaakmeer et al., 1994; Geervliet et al., 1997), is strongly enhanced in roots of belowground-infested *Brassica* plants (Blaakmeer et al., 1994; Geervliet et al., 1997; Ferry et al., 2007; Soler et al., 2007). This suggests that root volatile “bouquets” may have a different composition from shoot VOC profiles. These differences may be related to differences in the performance of these compounds in soil environments. Properties such as polarity, boiling point, and solubility determine the degradation, adsorption to soil particles, and the distance over which a compound can disperse through soils, which in turn are important factors for the perception by soil biota. At present, the diversity of herbivore-induced VOCs released by aboveground plant organs appears to be greater than that in roots. It must be noted, however, that there still is a paucity of data on root specific VOCs, which leads to a bias, underestimating VOCs from roots.

In addition to local VOC responses, root herbivores also may induce systemic responses in shoots. The activities of root herbivores not only affect aboveground herbivores that are ovipositing and feeding on the leaves of the same plant (Bezemer et al., 2004; Anderson et al., 2011), but also alter the behavior of organisms at higher trophic levels—such as parasitoids and predators—foraging above ground (Rasmann and Turlings, 2007; Soler et al., 2007, this issue). The effect on aboveground higher trophic levels can either be mediated through changes in the host plant quality elicited in root-induced plants, such as proteinase inhibitors and the accumulation of secondary metabolites, or *via* changes in the volatile bouquets of root-induced plants that render these plants less attractive (Rasmann and Turlings, 2007; Soler et al., 2007). Changes in VOC emissions due to root herbivory can be detected in both belowground and aboveground tissues. Such root-induced changes in VOC emissions possibly can be exploited as indicators of root damage by herbivores without harvesting the plant. In order to do so, we need sensitive and non-invasive techniques that are capable of detecting minute changes in VOC emissions. Proton-transfer-reaction mass spectrometry (PTR-MS) is an on-line technique that allows the sensitive assessment of plant VOCs in real-time. In this review, we discuss the potential, possibilities, and pitfalls of using PTR-MS for the non-invasive and on-line analysis of VOCs induced by root herbivores in comparison to more traditional techniques applied in VOC research.

VOC Analysis Using GC Platforms

Plants emit substantial amounts of their assimilated carbon as VOCs: Up to 10 % of their carbon assimilation can be released in this way (Peñuelas and Llusià, 2004). These emissions mainly consist of isoprene, a short chain (C5) hydrocarbon. The highly diverse class of higher isoprenoids (>30,000 different structures are described to date; Connolly and Hill, 1991) contributes smaller proportions, and their emission rates are often correlated with biotic and abiotic stressors. In the early days of chemical ecology, around three decades ago, the ability to investigate gaseous emissions from plants focused on the major peaks in the chromatogram. With the progress of analytical technologies, we are more and more approaching whole metabolome analyses, which is important, since minor compounds in the background of a complex volatile blend can contribute significantly to the biological activity of that blend (Mumm and Hilker, 2005; van Dam and Poppy, 2006).

The emission rates of plant VOCs usually are very low, ranging from a few nanograms to micrograms per gram plant dry weight, released per hour. At present, reported emission rates are particularly difficult to compare. In the chemical ecology literature, emission values are either presented in relation to plant weight (dry or fresh), or as relative emissions (e.g., Geervliet et al., 1997; Pierre et al., 2011b). This is, most likely, due to the fact that it would require many authentic standards to properly quantify each VOC in GC-MS analyses, and these are often difficult to obtain. Frequently, it also is a problem of units that prevents direct comparisons between published studies, especially between GC-MS and PTR-MS analyses.

A conversion of the unit commonly used for VOC emissions, in $ng \cdot g^{-1}$[plant weight]$\cdot h^{-1}$, into mixing ratios (in parts per billion volume, a common non SI-unit to report PTR-MS results) will give us this opportunity. We can approximately convert emissions by the following formula[1]:

$$\mathrm{Emission}_{ppbv} = \frac{\mathrm{Emission}_{g \cdot g^{-1}[DW] \cdot h^{-1}} * 24.5 \frac{\mathrm{l}}{\mathrm{mol}} * \mathrm{m}_{plant}}{\mathrm{M}_r * \mathrm{V}_{air}} \quad (1)$$

Here, we give an example of this conversion for green leaf volatiles (GLVs), which are one of the most widespread VOC classes in the plant kingdom, at least above ground (Table 1). Because of the molecular weight, which has to be taken into account for the conversion, each compound contributes different ratios to the total emission, depending on the unit the value is described with. The GLV (*Z*)-3-Hexenol, for example, was emitted at a rate of 153.6 $ng \cdot g^{-1}$ [dw]$\cdot h^{-1}$, which is 43 % of all GLVs in the herbivore treatment, but when expressed as a mixing ratio (ppbv), the same compound constitutes 53 % of all GLVs (Table 1). Depending on the context, for example for insect physiology, the values might be biologically more informative when presented on a number-of-molecule basis, whereas in atmospheric chemistry the gram-based units might be preferred.

Due to low emission rates, the VOCs sampled from the plant headspace usually need to be pre-concentrated on adsorbents before they can be analyzed on gas chromatography (GC) platforms. Most commonly, plant VOCs are sampled on tubes filled with polymer materials, such as Tenax, Porapaq, Carbopack, and charcoal, or on solid phase micro-extraction fibers (SPME; D'Alessandro and Turlings, 2006; Tholl et al., 2006; Birkett, 2010). In view of this necessity to preconcentrate the sample before analysis, the procedure involves collection periods in the range of minutes to hours, which prevents highly time-resolved measurements of VOC emissions. Additionally, the sampling procedure may cause contaminations to be introduced when solvents are used to elute VOCs from the tubes before injection on the GC. With other sampling techniques this can be avoided. Using direct thermodesorption (TD) tubes, the VOCs are thermally desorbed from the packing material and transferred directly to the GC injector port in the gaseous phase (e.g., Pierre et al., 2011b) In both cases, however, the relatively high temperatures, essential for rapid desorption or evaporation of the solvent in the injector port, may cause the VOCs to breakdown or to be converted into other components (de Kraker et al., 1998). Certain VOCs, such as sabinene and α-pinene, also degrade to some extent as a result of reactions with the adsorbent surface (Rothweiler et al., 1991; Coeur et al., 1997). Moreover, depending on the packing materials of the sampling tubes, selective breakthrough of certain compounds, such as isoprene and other short-chain hydrocarbons, may occur, which makes the analysis less quantitative for these compounds (Dettmer et al., 2000). By contrast, PTR-MS has the potential to sample VOCs on-line and with high sensitivity (pptv), without the need for pre-concentration, thereby avoiding many of the above-mentioned drawbacks. Additionally, the instrument operates at much lower temperatures (around 50°C) which reduces the formation of chemical artifacts (Hansel et al., 1995).

Proton-Transfer-Reaction Mass-Spectrometry (PTR-MS)

About 15 years ago, PTR-MS emerged as a powerful tool for monitoring VOCs. Whereas conventional MS technology is often based on electron ionization (EI), which results in extensive fragmentation providing rich ion fragments, PTR-MS relies on chemical ionization (CI), a soft ionization method with few or no ion fragments in the mass spectra. A detailed description of the PTR-MS technology has been published elsewhere (Hansel et al., 1995; de Gouw et al., 2003; Boamfa et al., 2004). Here, we briefly outline the main characteristics of the PTR-MS technology in so far as they are essential to be able to evaluate its opportunities and limitations for plant VOC analyses.

In PTR-MS, a neutral molecule is ionized *via* a CI reaction with H_3O^+. The ionized molecules typically form a protonated molecular ion $[M+H]^+$, in which M is the molecular mass of the parent molecule. Water or a mixture of water and helium is introduced and the H_3O^+ ions are produced by a, mostly hollow, cathode discharge in the primary ion source (Fig. 1, no. 1; Boamfa et al., 2004). Thereafter, the H_3O^+ ions enter the reaction chamber, the so-called drift tube (Fig. 1, no. 2), where they are driven by a homogenous electric field and will interact with the trace gas mixture that enters directly *via* an inlet at low gas flow rate (~ 0.5 l/h). Typically, only molecules with a proton affinity higher than that of water (>166.5 kcal mol^{-1}) will be ionized by proton-transfer-reactions with H_3O^+ ions. Organic compounds such as aldehydes, ketones, alcohols,

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[1] The formula assumes an ideal gas at T=25°C and P=1 atm. For more precise conversions, parameters such as the true temperature during sampling, need to be accounted for. The molar volume of an ideal gas at these conditions is 24.5 $l \cdot mol^{-1}$. **$\mathbf{M_r}$**—relative molecular mass, **DW**—dry weight, **$\mathbf{m_{plant}}$**—total plant dry weight of sampled plant part, **$\mathbf{V_{air}}$**—total volume of air sampled.

Table 1 Conversion of units commonly found in the literature for plant VOC emissions with an example of gypsy moth-induced green leaf volatiles (GLVs) of poplar (*Populus trichocarpa*), adapted from Danner et al. (2011)

Compound	M_r[a]	Emission [ng · g^{-1} DW[b] · h^{-1}]		Emission [ppbv[c]]	
	[g · mol^{-1}]	Control	Herb.	Control	Herb.
(*Z*)-3-Hexenol	100.2	9.4	153.6	0.16	2.61
(*Z*)-3-Hexenylacetate	142.2	19.1	153	0.22	1.83
Hexyl acetate	144.2	1.1	44.7	0.01	0.53

[a] M_r—relative molecular weight

[b] DW—dry weight

[c] ppbv—parts per billion volume

oxygenated aromatic and aliphatic compounds will be readily protonated (Warneke et al., 2003; Hartungen et al., 2004; Wisthaler et al., 2005; de Gouw and Warneke, 2007).

In addition to the normal proton-transfer-reaction, the H_3O^+ and $[M+H]^+$ ions can cluster with water molecules in the drift tube, complicating the interpretation of mass spectra. Since the proton affinity of the clusters is higher than that of water, the proton-transfer-reaction with a water cluster will be favored. An important part of the instrument that serves to reduce problems of cluster formation is the collision dissociation chamber (Fig. 1, no. 3). In this intermediate chamber, the cluster ions that leave the drift tube dissociate into a neutral moiety and the initial, protonated trace gas molecule $[M+H]^+$. Cluster formation can be reduced further by adapting the reaction conditions of the PTR-MS instrument for this purpose (2 mbar pressure and 120–140 Td field strength in the drift tube).

As described above, the proton-transfer results in few or no fragment ions for most trace gas compounds. Despite this general rule, fragment ions are still detected in the mass spectra for certain compounds, increasingly more with higher kinetic energy in the drift tube (Maleknia et al., 2007). Above all, the fragmentation pattern depends on the structure of the molecules. For example, alcohols break down easily and lose a water molecule *via* dehydration, whereas acetaldehyde or acetone is less likely to dissociate (Boamfa et al., 2004). Although there are extensive resources for EI fragmentation patterns [e.g., National Institute of Standards and Technology (NIST, USA) and Wiley (West Sussex, England)], these spectral libraries cannot be applied as a reference to PTR-MS, in view of the dissimilar ionization methods. Consequently, one needs to determine the fragmentation behavior of the VOCs under study either from the literature, or by reference measurements with authentic standards.

Another aspect to be considered is the back diffusion of air from the drift tube into the ion source, which leads to contaminant ions, such as NO^+ and O_2^+ (de Gouw and Warneke, 2007). PTR-MS of plant VOCs requires the amount of these ions to be reduced, because they transfer their charge to most VOCs without adding a proton, which complicates the identification of compounds. However, higher levels of NO^+ and O_2^+ ions may also be beneficial for detecting specific compounds, such as sulfur-containing glucosinolate breakdown products (Crespo, 2012).

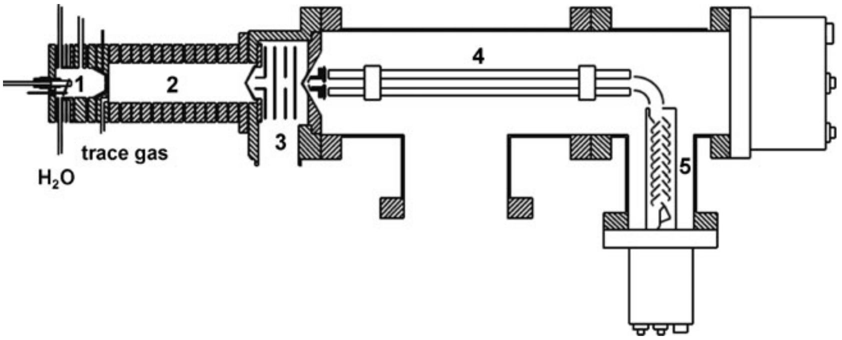

Fig. 1 Schematic representation of a typical PTR-MS. The instrument consists of an ion source (1) in which H_3O^+ primary ions are produced, a drift tube (2), where the trace gases from samples are ionized by the proton-transfer reaction with H_3O^+ ions, a collisional dissociation chamber (3), where cluster molecules dissociate, and the detection unit, where ions are mass filtered with a quadrupole mass filter (4) and quantified by a secondary electron multiplier (5)

Quantification and Identification of VOCs

In PTR-MS, regular calibration with an authentic gas mixture is a prerequisite for reliable quantification of trace gases, for example, drift tube humidity can vary, which has an impact on the drift tube reactions. A typical example of a calibration gas mixture consists of acetaldehyde, acetone, isoprene, benzene, toluene, xylene, and α-pinene (covering molecular masses from 32 amu to 136 amu), each at a concentration of 1 ppmv (parts per million volume, ±5 %). The calibration factors obtained for the fixed set of compounds in the certified gas mixture can be used to calculate the calibration factors of other compounds, by taking into account their collision rate constants, transmission efficiency factors, and fragmentation ratios. In this way, ion intensities (expressed as normalized counts per second, ncps) can be converted to absolute concentrations as gas mixing ratios (parts per billion volume, ppbv). PTR-MS can operate in two modes, namely the full mass scan and selective ion monitoring (SIM). The first scans the relative abundance of all detectable masses, and should be regarded as a fingerprint of a given trace gas sample (Steeghs et al., 2004). In contrast, the SIM mode is suitable for recording temporal changes in concentrations of specific trace gas molecules, pre-selected by their mass-to-charge ratios.

The major drawback with PTR-MS remains the identification of compounds, which is notoriously difficult, as each detected mass can either be associated with parent molecules, fragments of parent molecules, and water clusters, or a combination of these. Therefore, the identification of compounds measured by PTR-MS is mostly tentative. Nevertheless, if several compounds with the same nominal mass must be considered as possible candidates in a gas mixture, several methods to distinguish between these compounds can be employed. For example, water clusters can be easily distinguished from compounds undergoing the usual proton transfer reaction by varying the field strength in the drift tube (E/N). Association processes with water are quite sensitive to higher collision energies (E/N), thus, if the intensity of a signal decreases with higher E/N, the signal is contributed by a compound associated with one or more water molecules.

In the same way, the abundance of stable isotopes can provide further information about the identity of a compound. The probability of ^{13}C incorporation into a molecule rises in a linear fashion with the number of carbon atoms in that molecule. For example, with the natural ^{13}C abundance of 1.1 %, a molecule containing 5 carbon atoms, such as isoprene (M=68) has a chance of 5.5 % to contain exactly one ^{13}C. With PTR-MS, isoprene is detected as $C_5H_9^+$ at m/z=69, however, this signal also can be attributed to a water–methanol cluster-ion, $CH_3OH\ (H_2O)H^+$. If the ratio between m/z=69 and its isotope at m/z=70 indicates a ^{13}C abundance close to the expected value for a 5-carbon compound (5.5 %) the signal measured at m/z=69 is more likely to be derived from isoprene. Additionally, in complex gas mixtures, such as the ones derived from plant headspaces or human breath, it is common practice to proceed along these lines for compound identification (Lindinger et al., 1998; Crespo et al., 2011). Additional information about the identity of the molecule species also can be obtained from any other element with stable isotopes. Examples are nitrogen with an isotopic ^{15}N/^{14}N ratio of 0.366 %, hydrogen with a ^{2}H/^{1}H ratio of 0.015 %, and sulfur with a ^{34}S/^{32}S ratio of 4.21 %. However, ion-trap-based PTR-MS with the ability to perform MS/MS, TOF-based PTR-MS with high mass resolution, or coupling of GC with PTR-MS, are the preferred options for the unambiguous identification of compounds (Joó et al., 2010).

Basically, there are two main issues associated with the identification of compounds. First of all, the signal of the parent and fragment ions (isobaric ions) from different compounds can be superimposed on one m/z in the spectra without the possibility of discrimination. This complicates straightforward identification of VOCs in complex mixtures. Moreover, compounds with different structures but the same molecular mass appear at the same m/z signal and cannot be distinguished with a quadrupole mass filter (e.g., different monoterpenoids). To overcome these limitations, several new technologies have been developed. Combining PTR-MS with a GC, in which the VOCs are first separated by their retention time in the GC and then detected one by one by PTR-MS, avoids the overlap of different compounds and fragments (Warneke et al., 2003). Proton-transfer-reaction ion-trap mass spectrometry (PIT-MS) is another promising development to differentiate between different compounds with similar masses (Steeghs et al., 2004). This technique has characteristics similar to those of the PTR-MS, except that an ion trap is used, instead of a quadrupole as a mass analyzer. In PIT-MS, collision-induced dissociation (CID) is performed inside the ion trap, allowing different compounds with an identical mass to be differentiated by their fragmentation pattern (MS/MS). This approach enables, for example, the identification of different terpenoids and their oxygenated derivatives. Very promising is the recent development of a high-resolution time-of-flight (TOF) based system, PTR-TOF-MS, which is able to distinguish between isobaric molecules and allows unambiguous identification based on exact masses (Blake et al., 2004; Ennis et al., 2005; Graus et al., 2010). In classical PTR-MS, only one type of precursor ion (H_3O^+) is commonly employed to ionize compounds. In addition, other ions such as NO^+ and O_2^+ can be produced in the ion source with the switchable reagent ions (SRI) technology (Jordan et al., 2009). These primary ions allow compounds with proton affinities lower than that of water (e.g., halogenated hydrocarbons) to be detected and isomeric compounds to be distinguished.

Applications of PTR-MS for Biological Research

Since its development, PTR-MS has found many applications in a wide range of fields, including medicine (Cristescu et al., 2011), environmental sciences and atmospheric chemistry (de Gouw and Warneke, 2007; Bamberger et al., 2010; Ruuskanen et al., 2011), food monitoring (Raseetha et al., 2011), monitoring for safety and security at the workspace (Hansel et al., 1995), VOC emissions from plants during various abiotic stress conditions (Gray et al., 2010; Ruuskanen et al., 2011), and, most importantly in the context of this review, in understanding the chemistry of plant-herbivore interactions (Schaub et al., 2010; Brilli et al., 2011).

The VOC emissions resulting from plant-herbivore interactions are highly complex and dynamic. PTR-MS offers the opportunity to follow these processes in real-time. It has proven extremely difficult to use conventional sampling techniques and GC platforms to follow the fast conversion processes taking place in the lipoxygenase pathway (LOX) immediately after leaf wounding. With PTR-MS, this process has been studied at a high time-resolution, which yielded new insight into the regulation of this pathway (Fall et al., 1999; D'Auria et al., 2007). PTR-TOF-MS enabled the timing of the enzymatic conversions in the LOX pathway to be elucidated in mechanically wounded *Dactlylis glomerata* plants (Brilli et al., 2011). The conversion processes were analyzed from the initial membrane breakdown, resulting in fast emissions of C6 aldehydes, until the somewhat slower conversion of the intermediate C6 alcohols into hexyl and hexenyl acetates. Mobile PTR-MS equipment also has been used to investigate the timing of herbivore-induced green leaf volatiles, monoterpenoids, and sesquiterpenoids in poplar trees in the field (Schaub et al., 2010) and to monitor VOC emissions from complex vegetations such as grasslands or forest canopies (Davison et al., 2008; Bamberger et al., 2010; Ruuskanen et al., 2011).

However, the examples above all relate to plant volatiles induced above ground. To our knowledge, only one study has investigated root VOCs by means of PTR-MS. This study analyzed VOC emissions of *in vitro* cultured *Arabidopsis* roots after infection with a pathogen, *Pseudomonas syringae*, and the aphid *Diuraphis noxia*. The infections induced several simple metabolites, such as acetic acid, acetone, and ethanol, and a single monoterpenoid, namely 1,8-cineole (Steeghs et al., 2004). Interestingly, GLVs were not found to be released by damaged *Arabidopsis* roots. As the roots were grown *in vitro,* however, the question remains how representative the herbivore-induced responses observed in this experiment are for plants that are growing in the soil.

Therefore, we present two examples of preliminary PTR-MS results on herbivore-induced root responses in *Brassica* species obtained with a custom-made PTR-MS described in detail in Boamfa et al. (2004). We monitored VOCs emanating from roots of potted turnip plants (*Brassica rapa* subsp. *rapa* var. Nancy) during infestation with a belowground herbivore, the larvae of the cabbage root fly (*Delia radicum*). The root headspace of infested and non-infested plants was sampled from a cuvette fitted around the base of the stem (Fig. 2). The two parts of the cuvette were sealed together with Terostat IX (Henkel, UK), a solvent–free, rubber-based sealant (Crespo, 2012) to prevent ambient air from entering. During measurements, an excess flow of hydrocarbon-free air into the cuvette was maintained, similar to a typical dynamic headspace collection setup (Tholl et al., 2006). The resulting mass scan (Fig. 3) shows that the intensities of several molecular masses are enhanced in root fly infested *B. rapa* roots, the identities of which were confirmed by Crespo (2012) by additional GC-MS analysis and PTR-MS measurements of authentic standards. The induced intensities were detected in several structurally related sulfides, some of which have been shown to be induced in more than one *Brassica* species after root fly feeding, and which are exploited as cues by parasitoids and predators (Ferry et al., 2007; Soler et al., 2007). The mass-charge ratios representing dimethyl disulfide (DMDS; $m/z=63$) and dimethyltrisulfide (DMTS; $m/z=95$) displayed considerable increases in emission rates due to herbivore feeding. In addition, we also found that the biosynthetically related compound methanethiol ($m/z=49$) was emitted at higher rates when root fly larvae were feeding. Previous GC analyses have not detected methanethiol, which might originate from the compound selectivity of the adsorbents that have been used for collection. Interestingly, we also found a considerable increase in $m/z=60$, which is related to glucosinolate breakdown products (Crespo, 2012). This is a typical characteristic of members of the *Brassicaceae* after tissue damage. After disruption of the cells, a separately stored enzyme (myrosinase) converts the glucosinolates that are stored in the vacuoles into toxic and volatile products, such as isothiocyanates and nitriles (Hopkins et al., 2009). After activation of this two-component defense mechanism, the volatile conversion products are detected in the headspace of damaged plants (Soler et al., 2007; Pierre et al., 2011b). Our preliminary

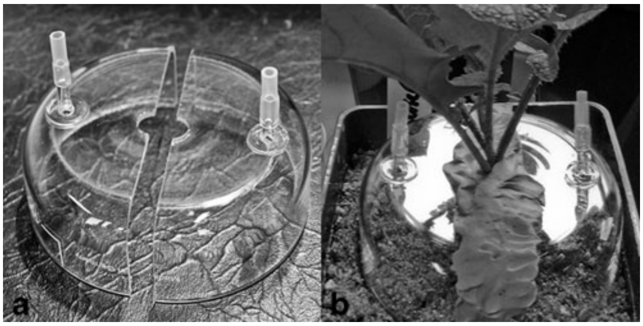

Fig. 2 Cuvette used for dynamic headspace collections from plant roots. **a** the cuvette which consists of two parts with an air in- and outlet, respectively. **b** cuvette fitted together and tightened with a rubber-based sealant

experiment shows that glucosinolate conversion products also emanate from roots, damaged by soil herbivores.

As another example, we monitored the induction of VOCs in *B. juncea* roots after infestation with *Delia radicum* in real-time and compared it to a control treatment (Fig. 4). We followed the emission of root VOCs for several hours in SIM mode, starting immediately after ten actively feeding second instar larvae were added to the roots. Based on the previous example, we chose to record specifically the masses which correlate to the three sulfides from the previous experiment and the mass 60, all of which already revealed differences between the treatments in scan mode (Fig. 3). Initially, we observed a low emission rate of only several ppbv for these compounds, which steadily increased with longer feeding times of the root flies (Fig. 4). In control plants, the VOC emissions remained at a very low level, which allowed a clear distinction between control and infested plants within a few hours after infestation. We suggest that further development of PTR-MS methods and sampling set-ups might provide us with the tools to correlate the intensities of the VOC emissions directly to the amount of herbivore damage in a quantitative manner. Possibly, the PTR-MS emission patterns can be used to assess the infestation level of root herbivores or to assess the time point when they stop feeding or start pupating by exploiting certain VOC related masses as non-invasive markers.

Conclusions

As outlined, PTR-MS has in the recent years opened an avenue for new insight into fast changing, highly dynamic processes involved in plant VOC emissions caused by plant-environment interactions. Here, we show that, due to its sensitivity and the ability to record real-time responses, PTR-MS is an excellent technique to non-invasively trace the feeding activities of cryptically feeding root herbivores by measuring VOC emissions from the root headspace. Certainly, PTR-MS also has its practical and technical limitations. Besides difficulties in linking masses without doubt to compounds, many quadrupole-based systems lack sensitivity in the higher mass range (above 120 amu), which is relevant for plant-herbivore interactions, as many biologically important compounds, such as several isothiocyanates or generally hemiterpenoids and sesquiterpenoids are difficult to detect. These shortcomings can be overcome partially by combining on-line sampling with PTR-MS and off-line GC-MS methods, or by use of high sensitivity PTR-MS instruments with mass analyzers, such as distinctive quadrupoles, triple quadrupole technology, ion trap, or time-of-flight, which can provide sensitivity also in the higher mass range (Tani et al., 2003; Kim et al., 2009). We expect that the innovative and fast-evolving field of MS technologies will result in further improvements regarding sensitivity and mass resolution. Consequently, PTR-MS-based technologies may soon approach detection limits even closer to the sensitivity of insect antennae. A recently developed sensor, for example, which uses antennae of Colorado potato beetles (*Leptinotarsa decemlineata*) or of jewel beetles (*Phaenops cyanea*), demonstrates that insect antennae are capable of detecting, for instance, the GLV (*Z*)-3-hexen-1-ol at around 1 ppmv and 1 pptv, for the two species, respectively. Compared to that, the detection limits in PTR-MS already are in a similar range of several parts per trillion volume, depending on the properties of the instrumentation.

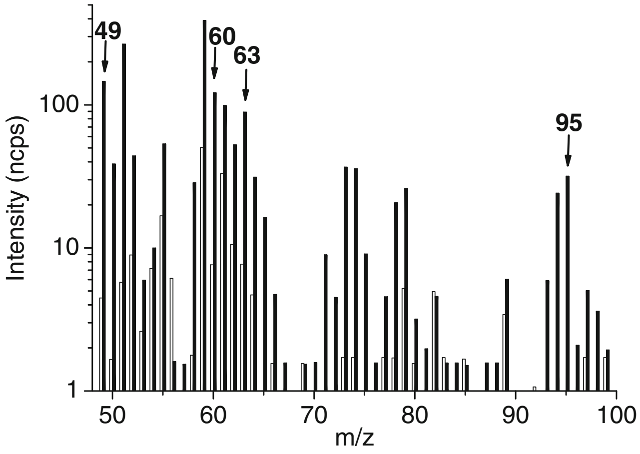

Fig. 3 Identification of enhanced signals at masses correlated to volatile organic compounds (VOCs) from *Brassica rapa spp. rapa Nancy* by PTR-MS (*scan mode*) after root herbivory by *Delia radicum* (*black bars*) vs. control plants (*white bars*). [ncps]—normalized counts per second, *m/z*=49—methanethiol, *m/z*=60—related to glucosinolate breakdown products, *m/z*=63—dimethylsulfide (DMS), *m/z*=95—dimethyldisulfide (DMDS)

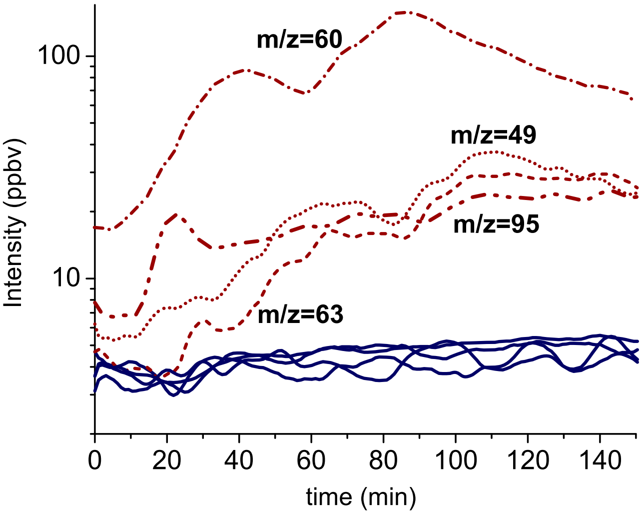

Fig. 4 Temporal dynamics of volatile organic compound emission from *Brassica juncea* after root herbivory by *Delia radicum* (*broken lines*) and a control without damage (*continuous lines*) by PTR-MS (SIM mode). *m/z*=49—methanethiol, *m/z*=60—related to glucosinolate breakdown products, *m/z*=63—dimethylsulfide (DMS), *m/z*=95—dimethyldisulfide (DMDS)

Finally, time-resolved and sensitive on-line sampling of root-induced volatiles with PTR-MS will certainly contribute to our understanding of the role of VOCs in belowground multi-trophic interactions. In particular, linking real-time responses in the emission of VOCs to immediate behavioral responses of herbivores, and to the higher trophic levels of parasitoids and predators will unravel further details of the VOC 'language' among plants and between plants and insects. This may be achieved by 'sniffing out' the VOCs in parallel with olfactometer assays, a prime example of which is the development of a six-arm olfactometer, simultaneously equipped with a VOC sampling unit (Turlings et al., 2004). In a similar way, coupling PTR-MS analyses with microbial bioassays may help to disentangle the impact of belowground plant VOCs on other soil organisms, such as pathogens and microbes (Effmert et al., this issue). In addition to that, new approaches in multivariate statistical analyses will facilitate discrimination of biologically meaningful information from noise contained in these increasingly complex mixtures (van Dam and Poppy, 2006; Jansen et al., 2010) with steadily increasing numbers of compounds due to the rising sensitivity of instrumentation. Last but not least, improving our methods for non-destructive plant VOC sampling from soil environments will complement the knowledge we have gained already from aboveground plant organs, with further discrepancies and similarities being discovered between the two compartments of plants living apart together. With a more complete perspective on plant VOCs and their biological roles, we might later be able to complement our current perceptions of plant defenses, adapted to a perspective of the whole plant.

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Novel Bioassay Demonstrates Attraction of the White Potato Cyst Nematode *Globodera Pallida* (Stone) to Non-volatile and Volatile Host Plant Cues

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Abstract Potato cyst nematodes (PCNs) are a major pest of solanaceous crops such as potatoes, tomatoes, and eggplants and have been widely studied over the last 30 years, with the majority of earlier studies focusing on the identification of natural hatching factors. As a novel approach, we focused instead on chemicals involved in nematode orientation towards its host plant. A new dual choice sand bioassay was designed to study nematode responses to potato root exudates (PRE). This bioassay, conducted together with a traditional hatching bioassay, showed that biologically active compounds that induce both hatching and attraction of PCNs can be collected by water extraction of incised potato roots. Furthermore, our results demonstrated that PCN also were attracted by potato root volatiles. Further work is needed to fully understand how PCNs use host plant chemical cues to orientate towards hosts. Nevertheless, the simple attraction assay used in this study provides an important tool for the identification of host-emitted attractants.

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Introduction

The potato cyst nematodes (PCNs) *Globodera pallida* and *G. rostochiensis* are among the most important pests of solanaceous plants. Infestation by PCNs occurs immediately after hatching when second stage juveniles (J2s) invade host plants roots where they retard their development. Retardation of root extension reduces water and nutrient uptake by the plant and decreases the yield of potatoes (Trudgill et al., 1998). In Europe, a 9 % loss in total potato yield is attributed to PCNs (Evans and Rowe, 1998). Crop rotation leading to slow depletion of nematode populations as well as breeding of resistant varieties are the most frequently employed strategies since the prohibition of nematicides in many European countries (Chitwood, 2003). Recent investigations have focused on new control strategies based on the use of bacteria that directly or indirectly affect the performance and survival of plant-parasitic nematodes (Tian et al., 2007).

In the last 20 years, effort has been directed to the isolation and identification of host-derived PCN egg hatching factors (Twomey, 1995; Perry and Gaur, 1996; Devine and Jones, 2000a, b; Ryan et al., 2000; Devine et al., 2001; Ryan and Jones, 2003, 2004; Ryan and Devine, 2005). The identified hatching agent from potato roots, solanoeclepin A (Schenk et al., 1999), has a complex polycyclic structure and has yet to be artificially synthesized (Hue et al., 2005). Consequently, commercial production of this compound is not likely in the near future. Thus, there is a need to develop other environmentally and economically sustainable strategies for the control of PCNs.

An alternate control strategy could be to interrupt the life cycle of PCNs during the larval stage. Similar to the control of insect pests (Cook et al., 2007; Witzgall et al., 2010), semiochemicals could be employed to prevent nematodes from finding host plants. To develop such strategies, a sound basic knowledge of PCN attraction by semiochemicals is needed. However, little is known about such mechanisms. It has been suggested that PCN relies on various chemical signals that are operant at different distances from the host (Perry, 2005). Volatile compounds that are able to diffuse through the soil are the most promising candidates as long distance attractants. For instance, CO_2 emitted at root level (Hinsinger et al., 2003) has been shown to attract numerous nematode species (Robinson, 1995). In contrast, water-soluble compounds may act at shorter distances from the host. It has been suggested that nematodes follow a chemical gradient of root cues to locate a suitable invasion site (Mende et al., 1998). A prerequisite for understanding nematode attraction is that candidate chemicals can be tested in a reliable bioassay that adequately mimics the physicochemical environment in the rhizosphere. The aim of this study was to design such a bioassay and infer the mode of biological activity of different extracts. We tested the influence of potato root exudates (PREs) and potato root volatiles (PRVs) as host plant attractants for PCNs.

Methods and Materials

Plant Material All experiments were performed using a potato cultivar (*Solanum tuberosum* cv. Maria) highly susceptible to PCNs. Seed potatoes harvested in the autumn were stored at 5°C in darkness over winter. In May 2009, potato tubers were placed in darkness at room temperature for pre-germination until the appearance of the first shoots, and they subsequently were exposed to light in the greenhouse for 2 wk. To obtain tubers with a single stem, one 5–10 mm green shoot was conserved on each tuber.

Potatoes were grown in a greenhouse in 1 l-plastic pots filled with silver sand (Askania, Silver sand 36, dried) and given a standard NPK 8:7:16 fertilizer (Weibulls, Trådgård, Hammenhög). Photoperiod was set at 12:12 hL:D and remained constant during the entire growth period. Plants were watered every second day with 500 ml of tap water.

Preparation of Nematodes Nematodes were obtained from colonies of *G. pallida* originally collected in Sundsvall (Northern Sweden) and maintained in experimental fields in Alnarp (Southern Sweden). Cysts were extracted from soil samples and hydrated in tap water for 4 to 5 d. During this period, cysts were washed daily by stirring and renewing the water in order to detach fungi that could develop on the surface of the cysts and to limit the risk of bacterial proliferation. Hatching of second stage juveniles (J2s) was induced by overnight soaking of cysts in a solution of potato root exudates (PREs) (prepared as described below). Second stage juveniles were harvested from the PRE solutions while cysts were filtered out and placed in fresh PRE solution. Beakers of PRE solution containing newly emerged J2s were kept in a fridge to facilitate sedimentation of nematodes and the removal of excess solution. One ml of sedimented solution containing most of the nematodes was transferred to 1 l of tap water. This procedure was repeated twice to ensure that nematodes were immersed in PRE-free water. Second stage juveniles were stored at 4°C until utilization. The storage time before nematodes were used did not exceed 3 d post-hatching.

Preparation of Potato Root Exudates (PRE) The collection method for PREs was adapted from Rawsthorne and Brodie (1986). Three wk-old plants were removed from pots and gently washed to remove sand particles. Roots were cut from plants, weighed, and soaked in 250 ml beakers of water wrapped in aluminum foil to keep the roots in darkness. Beakers containing roots were kept at 4°C for 24 h when PREs were collected. PRE extracts were pooled, and their pH adjusted to 6.5 (i.e., the pH of the soil in which the potato plants are grown under natural conditions) by addition of a solution of 37 % hydrochloric acid 37 % (AR grade, Sigma Aldrich) diluted with water to 2 %. Potato root exudates were used in bioassays within 6 h of collection to limit microbial modification of their biological activity. Root weight (g) per liter of solution was used as a proxy unit for PRE concentration.

Sand Bioassay Small 6 mm deep arenas, constructed from poly-(methyl-methacrylate), (PLEXIGLAS®, Fig. 1), were used to assess the responses of J2s responses to chemical stimuli. Each arena was subdivided into three sections. Stimuli were applied in one side of an arena by wetting silver sand with the test solution (e.g., PRE) followed by compression of the sand. This side of the arena is hereafter referred to as the stimulus side (S-side). The centers of the arena and the control side (C-side) were prepared similarly, but the sand was moistened using water only. Silver sand has been used successfully in other nematodes bioassays (Rasmann et al., 2005; Ali et al., 2010; Dalzell et al., 2011) and was chosen as medium because it allows good nematode mobility. Furthermore, this medium limits the influence of biotic factors such as rhizobacteria or other microorganisms occurring in soil of natural PCN habitats. The weight of sand and the weight of solution added to each arena were measured to ensure a constant moisture content of 20 % (w/w). Stimulus and control sides were switched between bioassays to limit possible positional effects caused by compounds remaining from previous experiments. Second stage juvenile nematodes were released in the center of the arena in a 10 μl droplet of water. The number of nematodes in this drop was not determined. Arenas were kept in darkness at room temperature, and nematodes were given 24 h to choose between different quantities of sand. After 24 h, the different sections of sand were lifted and removed from the arenas with a razorblade and a spatula. The J2s were recovered by re-suspension and separation in 5 ml of water (in 12 ml vials). The supernatant above each section of sand was poured into a counting-chamber for determination of the number of J2s under a Leitz stereomicroscope. Nematodes recovered from the S-sides of arenas were considered to have been attracted, nematodes in the central section were considered to have expressed no-choice, and nematodes in C-sides were considered not attracted to potato metabolites. Since the number of J2s inoculated into an arena was not known, the proportion of J2s found in the different sections was calculated based on the total number of nematodes recovered.

Hatching and Dose Response Bioassays Hatching bioassays were performed using PREs at different concentrations, prepared by dilution of an 83 g roots/l PRE solution. Fifty cysts were immersed in PRE (pH 6.5) at concentrations of 0, 14, 35, and 83 g roots/l, respectively. After incubation for 24 h, the J2s emerged were counted, as described above. Solutions of PREs at concentrations of 0, 7, 14, 35, and 83 g root/l were used also for attraction bioassays (using sand arenas as described above).

Influence of pH of PRE Solutions A solution of PRE of 80 g root/l was divided into four aliquots. Aliquots were pH adjusted to 4.5, 5.5, 6.5, and 7.5, respectively, by addition of a 2 % solution of HCl. The attractiveness of these solutions then was assayed in the sand arenas.

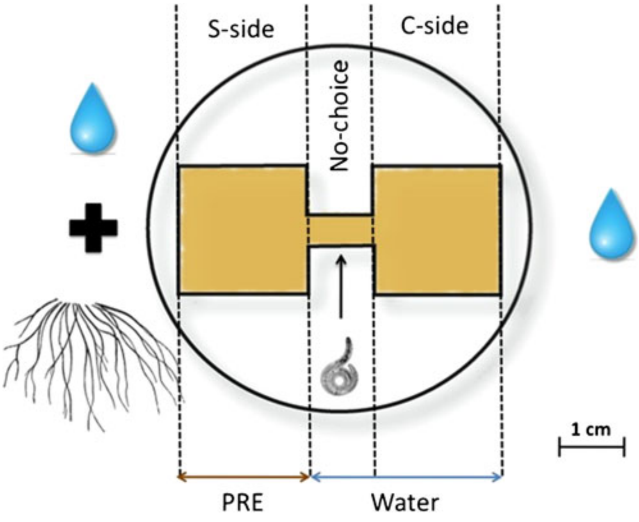


Fig. 1 Design of the bioassay: The arena is filled with moistened sand and is subdivided in three parts: the stimulus chamber (left, "S-Side"), the center where *Globodera pallida* second stage juveniles (J2s) are released, and the control chamber (right, "C-side"). At the end of the test, nematodes found on the stimulus side are considered as attracted, the ones in the center as non-choosing, and the ones on the right side as non-attracted

PRE Diffusion Rate The influence of stimulus diffusion through the sand arena on nematode responses was assessed by using a PRE solution of 80 g roots/l (pH=6.5) and staggered release of nematodes. The PCNs were released into arenas at 0, 12, 24, 36, and 48 h after they had been packed with sand and PRE solution was administered. Bioassays were allowed to run 24 h before J2s were recollected from the different sections of the arenas and counted as described above.

Influence of Freeze Drying of PRE Solutions To determine whether the non-volatile or volatile fraction of PRE was biologically active, a solution of 80 g roots/l was prepared and split into two fractions. One unaltered fraction was tested against nematodes in sand arenas ('crude PRE' contains volatile components) and the other was freeze dried and dissolved again in the same volume of ('freeze dried PRE' residue contains non-volatile components). Crude and freeze dried PRE solutions were adjusted to pH 6.5 before being used.

Collection and Testing of Potato Root Volatiles (PRV) Potato roots were washed and allowed to dry on tissue paper before being placed in a 500 ml glass jar with two outlets. Charcoal filtered air was drawn into the jar via one of the outlets, and volatile compounds trapped on a filter containing 30 mg of Super Q (Alltech Associates Inc.) attached to the other outlet. Air was drawn through the jar at the rate of 250 ml/min.

Volatiles were collected in darkness over a 24 h period. Volatiles were eluted with 300 μl of methanol (Labscan, Malmö, Sweden). The eluent was concentrated to 30 μl in glass vials under a fume hood. Concentrated extracts were sealed in glass capillaries and stored at −80°C until needed. Methanol was used in preference to dichloromethane or pentane due to its miscibility in water. Thus, the methanolic extract of potato root volatiles (PRVs) could be applied as stimulus in a way similar to the experiments with aqueous PRE extracts. Moreover, preliminary bioassays showed that pentane was strongly repellent to PCNs (approximately 90 % of J2s remained in the center of an arena when pentane was applied to surrounding sides; Farnier, pers. obs.). Hence, 20 μl of methanolic PRV solution were diluted in the same volume of water as was used to moisten the sand in the bioassays with PREs. Twenty μl of methanol also were added to the sand of the C-side of arenas as a procedural control. The pH was again adjusted to 6.5.

In addition, we studied the effect of the solvent alone on nematodes. Second stage juvenile responses in presence of

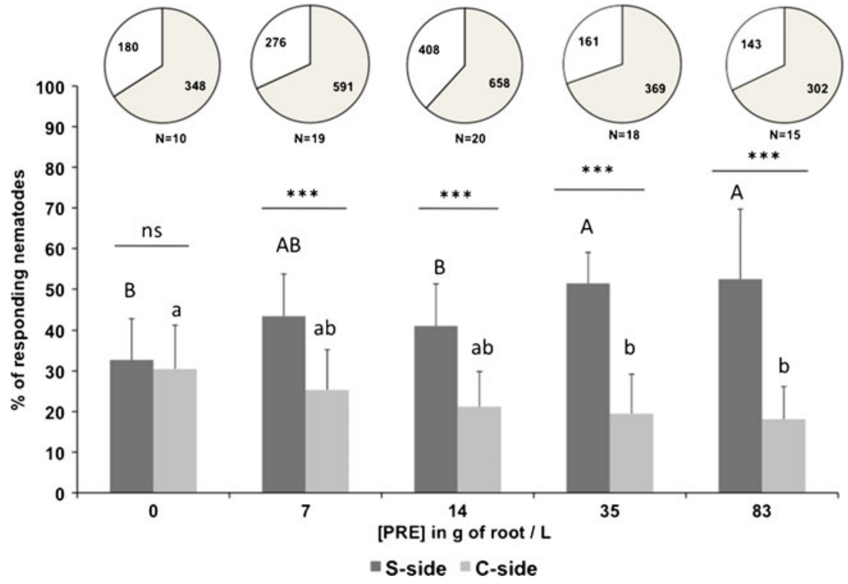


Fig. 2 Response of *Globodera pallida* second stage juveniles (J2s) to different concentrations of PRE in sand arenas. *N* is the number of replicate for each treatment. Shaded area of pie chart shows the total number of J2s in control and stimulus section, and the empty area shows the number of non-responders in the centre area of the bioassay arena. Bars show percent nematodes (±SD) in stimulus and control section. Asterisks show differences between the percentage of nematodes found in the stimulus and control sides within each treatment (paired Student's*t*-test, $P<0.001$). Capital letters represent statistical levels of differences between treatments in S-side ($P<0.05$) while small letters represent those in C-side ($P<0.05$); (One Way ANOVA followed by Tukey's test)

the amount of methanol used in tests performed with PRVs were assessed by applying on one side a 20 µl spike of methanol in the water used to humidify the sand of the S-side, and only water in the C-side.

Results

Attraction Bioassays Potato root exudates significantly influenced the number of nematodes found on each side of the arena at all concentrations tested (*paired Student's t-test,* $P< 0.001$; Fig. 2). On average, 50 % (and at the highest concentrations up to 80 %) of J2s preferred the side of the arena where PRE had been applied. An interaction between attraction responses at different concentrations also was observed (*one-way ANOVA,* $F_{4,68}=10.48$, $P< 0.05$). Within concentrations tested, the difference between the number of attracted and non-attracted nematodes was stronger with increasing concentration of PRE (*Tukey's post hoc* test*,* $P< 0.01$). The proportion of responding J2s (see pie charts in Fig. 2) was not significantly influenced by the concentration of PRE applied.

Fig. 3 Number of *Globodera pallida* second stage juveniles (J2s) hatched from 50 cysts after 24 h of exposure to different concentrations of PRE. *N* represents the number of replicates per treatment; errors bars represent the standard deviation. Letters above the columns represent the different statistical levels ($P<0.05$) between the treatments (One Way Anova, *post-hoc* Tukey's test)

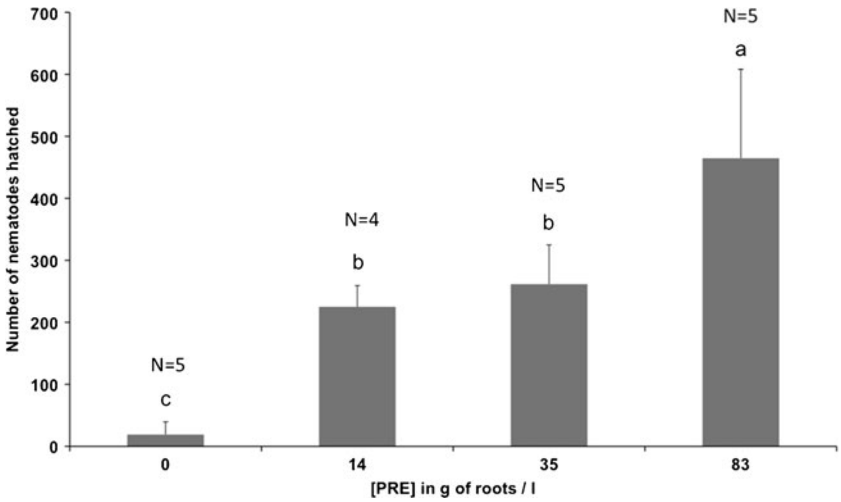
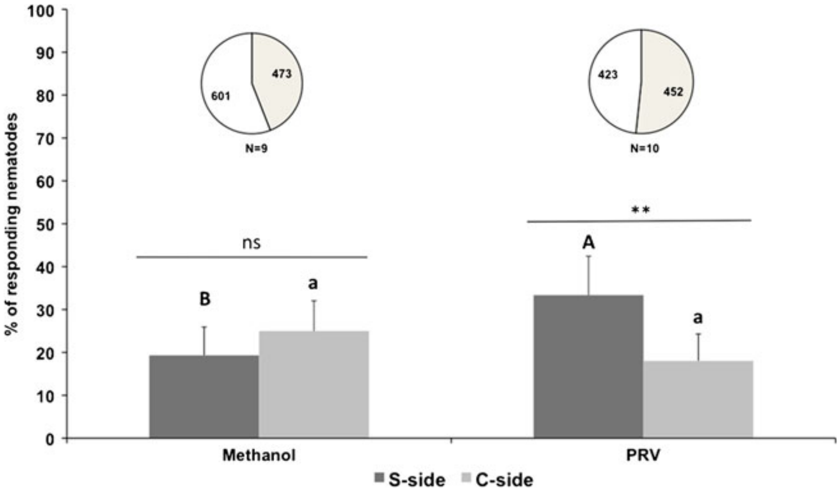


Fig. 4 Responses to crude PRE and freeze-dried PRE of *Globodera pallida* second stage juveniles (J2s) in sand arena. Legend explained in Fig. 2. Asterisks represent the levels of significance (paired-*t*-test, $P<0.001$) between the percentage of J2s found in C-side and S-side within each treatment. Capital letters show the differences for the S-Side between treatments (unpaired Student's *t*-test, $P<0.01$) and lower case letters those in the C-side (unpaired Student's *t*-test, $P<0.01$)



Hatching Bioassays The number of J2s hatching was highly influenced by the concentration of PRE [Fig. 3; One way ANOVA, *F (3,15)=24.09, P< 0.001*]. Hatching was increased at the lowest concentration of PRE used, i.e., 14 g roots/l (Fig. 3; *Tukey's post hoc* test*, P< 0.05*). Hatching increased proportionally according to PRE concentration (*Tukey's post hoc* test*, P< 0.001*).

Effect of pH and Diffusion Time on Attraction Neither pH (4.5-7.5) nor diffusion time (0–48 h) significantly influenced the responsiveness or orientation of J2s (data not shown).

Effect of Freeze-Drying of PRE on Attraction While crude PRE attracted J2s, freeze dried extract did not [*t (17)=3.582, P< 0.001;* Fig. 4]. Significantly higher numbers of J2s chose the C-side of arenas when the S-side had been treated with freeze dried PRE [*t (17)=3.244, P< 0.001*].

Response to PRV Although no repellency caused by the presence of methanol could be observed, J2 responsiveness was significantly lower in these experiments than in those conducted with PREs [compare Figs. 2 and 5; *F (2,27)= 15.56, P< 0.001*]. Nevertheless, more J2s were attracted by the methanolic extract of PRV than by methanol [*t (14)= 3.536, P< 0.01*) or by water (*t (7)=3.530, P< 0.01*]. No significant difference in the responsiveness of the J2s was found within this experiment (see pie charts in Fig. 5).

Discussion

Nematode behavior, and especially attraction to hosts, has been the subject of many studies each employing different bioassays. A key component for a successful bioassay is the medium provided for nematode dispersion and stimulus diffusion. Agar and sand are among the most frequently

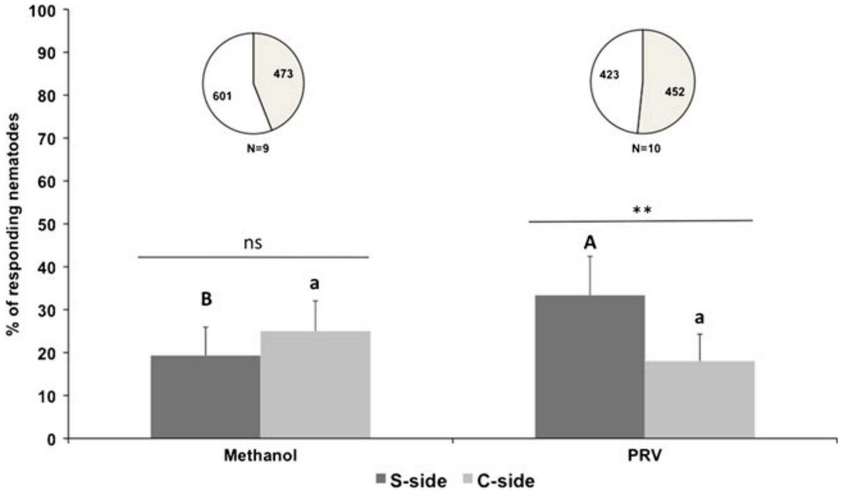


Fig. 5 Responses of *Globodera pallida* second stage juveniles (J2s) to methanol in a water background and to samples of PRV in a background of water and methanol. Legend explained in Fig. 2. Asterisks represent the levels of significance (paired-*t*-test, $P<0.001$) between the percentage of J2s found in C-side and S-side within each treatment. Capital letters show the differences for the S-Side between treatments (unpaired Student's *t*-test, $P<0.01$) and lower case letters those in the C-side (unpaired Student's *t*-test, $P<0.01$)

used media (Robinson, 2000). Agar allows good dispersion of nematodes and permits tracking of their movements. Sand provides a more realistic medium for both nematode movement and stimulus diffusion (Rasmann et al., 2005; Ali et al., 2010; Dalzell et al., 2011). Our study used sand and a simple compartmentalized arena to reliably bioassay PCN responses to different chemical stimuli. In combination, our approach provides a promising new tool for future studies on the same topic.

It previously has been suggested that the sensory organs of nematodes are not functional prior to hatching, which infers the existence of distinct host cues involved in hatching and attraction (Perry and Gaur, 1996). Our results confirm that aqueous extracts of potato roots elicit hatching of PCNs.

Our results suggest that pH does not influence PCN orientation towards host plants. The absence of any significant effect of pH on the responsiveness or orientation of J2s suggests that gradients in hydrogen ions established only over very short distances (about 1 mm) from the roots (see Rao et al., 2002) are unlikely to play a key role in host attraction. We, therefore, concur with Perry and Aumann (1998) who found that pH is unlikely to be a determinant in host plant localization by PCNs. Hence, plant metabolites probably are more plausible to mediate the attraction and explain the host specificity of PCNs than abiotic factors in the root microenvironment.

We hypothesized that biologically active compounds involved in the attraction of PCNs to host plant roots should be extractable in water, i.e., the likely natural carrier liquid in the soil environment. The effectiveness of the aqueous PRE as an attractant and catalyst of hatching for PCNs provides support for this hypothesis. We also found that the orientation of J2s was not influenced by the time the extract was given to diffuse throughout an arena. This finding suggests that the active compounds might be volatile. The loss of biological activity through freeze drying indicates that some host cues are indeed volatile. Compounds such as terpenoids, found in the PRV fraction (data not shown), can be present simultaneously in vapor and water phases (Fichan et al., 1999). Volatile compounds such as monoterpenes and sesquiterpenes are lost during freeze-drying (Abascal et al., 2005). Some nematodes are known to possess sensory neurons able to detect volatile compounds (Bargmann et al., 1990; 1993). The fact that attraction bioassays with PRVs showed a significant attraction of J2s confirms that some attractant metabolites are volatile. Taken together, our bioassays show that both PREs and PRVs are attractive to J2s, but the question remains whether the same chemical entities are responsible for attraction. Since nematodes have been shown to respond to both volatile and non-volatile compounds, it cannot be excluded that several compounds may be involved in the attraction and partly present in volatile and non-volatile fractions or eventually in both simultaneously (Bargmann et al., 1990).

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Substrate Temperature Constrains Recruitment and Trail Following Behavior in Ants

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Abstract In many ant species, foragers use pheromones to communicate the location of resources to nestmates. Mass-recruiting species deposit long-lasting anonymous chemical trails, while group-recruiting species use temporary chemical trails. We studied how high temperature influenced the foraging behavior of a mass-recruiting species (*Tapinoma nigerrimum*) and a group-recruiting species (*Aphaenogaster senilis*) through pheromone decay. First, under controlled laboratory conditions, we examined the effect of temperature on the trail pheromone of both species. A substrate, simulating soil, marked with gaster extract was heated for 10 min. at 25°, 35°, 45°, or 55 °C and offered to workers in a choice test. Heating gaster extract reduced the trail following behavior of the mass-recruiters significantly more than that of the group-recruiters. Second, analyses of the chemicals present on the substrate indicated that most *T. nigerrimum* gaster secretions vanished at 25 °C, and only iridodials persisted up to 55 °C. By contrast, *A. senilis* secretions were less volatile and resisted better to elevated temperatures to some extent**.** However, at 55 °C, the only chemicals that persisted were nonadecene and nonadecane. Overall, our results suggest that the foraging behavior of the group-recruiting species *A. senilis* is less affected by pheromone evaporation than that of the mass-recruiting species *T. nigerrimum*. This group-recruiting species might, thus, be particularly adapted to environments with fluctuating temperatures.

Keywords Ant communities · Trail pheromone · Temperature · Competition · *Tapinoma nigerrimum* · *Aphaenogaster senilis*

Introduction

Communities of ants, like those of many other organisms, are often characterized by the diversity of coexisting species. It is, therefore, fundamental to understand the factors that allow species coexistence at the community level (Andersen, 2008). Niche partitioning is a well-known mechanism that mediates competitive exclusion (Albrecht and Gotelli, 2001). Co-occurring species might differ in their

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nesting requirements (Torres, 1984), diet (Cerdá et al., 1998b; Sanders and Gordon, 2003; Blüthgen and Fiedler, 2004; Lebrun, 2005), foraging schedules, and/or resistance to environmental stress (Savolainen and Vepsäläinen, 1989; Hölldobler and Wilson, 1990; Cros et al., 1997; Retana and Cerdá, 2000). In environments with fluctuating temperatures, species with different thermal tolerance tend to segregate temporally in their foraging activity. In Iberian Mediterranean communities for instance, the two co-occurring species *Tapinoma nigerrimum* and *Aphaenogaster senilis* compete for arthropod corpses (Cerdá et al., 1998b), differ in their thermal preferences (Cerdá et al., 1998a), and field observations show that during the hot summer days, the former tends to forage at night while the latter is more abundant during the day (Cros et al., 1997).

In ants, foraging behavior can vary from being more individual to more social (Traniello, 1989). Although a few species forage individually without communicating food location to nestmates (e.g., *Cataglyphis spp.*, Wehner et al., 1983, Lenoir et al., 1990, 2009; or *Pachycondyla*, Fresneau, 1985), the great majority of ants rely on recruitment to exploit food sources (Wilson, 1971). Recruitment processes can involve a combination of cues ranging from physical contact, stridulation, and pheromones (Hölldobler and Wilson, 1990). In many species, scouts, who search the environment for food, deposit a pheromone trail upon food discovery. This pheromone trail communicates information about resource location to nestmates. In a self-amplifying process, recruits often become recruiters as they contribute to trail reinforcement. Recruitment behavior can be classified according to the complexity of communication it requires (Wilson, 1971; Beckers et al., 1989). In group recruitment (e.g., *Camponotus socius,* Hölldobler, 1971; Kohl et al., 2001), a small group of recruits follows a recruiter while it returns from the nest to the resource. By contrast, mass-recruitment (e.g., *Lasius niger,* Beckers et al., 1992) does not involve a leader (Bonabeau et al., 1998): it implies independent trail-laying and trail-following behavior. Both group and mass recruitment allow the adjustment of the collective response to a given environment (Detrain and Deneubourg, 2002; Cerdá et al., 2009). On the one hand, group recruitment permits the rapid mobilization of nestmates to exploit a wide range of food items (Cerdá et al., 2009). On the other hand, mass recruitment seems particularly efficient for longer lasting resources. By recruiting a huge number of workers to food sources, mass recruiting species are able to monopolize resources against competitors (Jaffe, 1980). In a competitive context, mass recruitment is, therefore, associated with behavioral dominance.

Temperature is an essential dimension of what Hölldobler and Wilson (1990) call the “temperature-humidity envelope” that determines the microenvironment favorable to the foraging activity of ants. In many communities, behaviorally subordinate species are more tolerant of stressful temperatures than their competitors (Cerdá et al., 1998a; Bestelmeyer, 2000; Lessard et al., 2009; Wittman et al., 2010). For example, in arid or semi-arid ant communities, subordinate species show behavioral (gaster raising in *Cataglyphis rosenhaueri*, Cerdá and Retana, 2000, Lenoir et al., 2009), morphological (long legs as shown by Stefan and Wehner (2012), larger workers are less subject to desiccation, Lighton and Feener, 1989), and/or biochemical (heat shock proteins synthesis, Gehring and Wehner, 1995) adaptations to arid environments. These apparent adaptations to stressful environments might result from character displacement, allowing these species to forage when ground temperature is suboptimal for their competitors. However, the constraints that prevent such thermal tolerance to evolve in dominant species remain unclear. Since behavioral dominance relies on the control of resources against competitors, we hypothesized that the efficiency of the recruitment process could be such a constraint.

As with many terrestrial invertebrates, ants maintain an intimate relationship with soil (see Vander Meer, 2012, this issue). In different Formicidae subfamilies, species that use chemical recruitment tend to forage at lower temperatures than species that do not (Ruano et al., 2000). This pattern might be due to the volatility of the trail pheromones. Indeed, high temperatures, by accelerating pheromone decays, limit trail-following behavior (van Oudenhove et al., 2011). The purpose of the present study was to determine whether high temperatures affected differently the foraging activity of ant species according to their recruiting system. In particular, we hypothesized that pheromone volatility limits recruitment behavior more in mass-recruiting species than in group-recruiting species. We tested this hypothesis with two widespread competing species in Iberian Mediterranean communities: *Tapinoma nigerrimum* and *Aphaenogaster senilis.* The former is a mass-recruiting species (Cerdá et al., 1989; Blight et al., 2010), whereas the latter uses group recruitment (Cerdá et al., 2009). To clarify the overall effect of high temperature on the foraging behavior of both species, we conducted a laboratory experiment in which gaster extracts were subjected to increasing temperatures. This approach allowed us to compare the rate at which trail pheromone dissipates as a function of ground temperature and independently of ant body temperature. Finally, we analyzed the composition of the gaster extracts and determined how they were altered by high temperatures.

Materials and Methods

Model Species and Study Sites *Tapinoma nigerrimum* is a highly polygynous, polydomous, and dominant species whose colonies contain tens of thousands of workers (Cerdá et al., 1989, 1997). Workers collect mainly aphid honeydew, and, to a lesser extent, arthropod corpses (Cerdá et al., 1989). Their trail pheromone originates from the pygidial gland located in the gaster (Pavan and Trave, 1958; Simon and Hefetz, 1991). *Tapinoma nigerrimum* is a heat-intolerant species: its Maximal Activity Temperature is 24 °C and its Critical Thermal Limit is 42 °C (Cerdá et al., 1998a). In contrast, *A. senilis* is a strictly monogynous, monodomous, and subordinate species whose colonies contain 1,300 workers on average (Boulay et al., 2007a). It is an opportunistic, omnivorous species that feeds on dead arthropods and a variety of vegetative items including petals and seeds (Boulay et al., 2007b). Workers use group-recruitment when food items are not transportable by individual foragers (Cerdá et al., 2009). The trail pheromone is composed of a mixture of alkaloids and hydrocarbons secreted by the Dufour and venom glands (Lenoir et al., 2011). It is a thermophilic species whose Maximal Activity Temperature and Critical Thermal Limit are 42 °C and 46 °C, respectively (Cerdá et al., 1998a).

Laboratory experiments were conducted in spring 2009 with ants freshly collected from the field in southern Spain (Doñana National Park), and transferred to artificial nests. For *A. senilis*, medium-sized queenright colonies (about 1,000 workers and abundant brood) were used. For *T. nigerrimum*, partial colonies (1,000–5,000 workers) with one or several queens were used. We employed 6 colonies of each species in our experiments. Artificial nests consisted of plastic boxes, the bottoms of which were coated with plaster to maintain humidity and simulate soil conditions. Test-tubes (2×20cm) that were half-filled with water and plugged with cotton also were placed in the nests to maintain humidity. Room temperature and humidity remained constant over the course of the experiment, at 25 °C±1 °C and 35 %±5 %, respectively. Ants were fed three times a week with meal worms (*Tenebrio molitor*). All experiments were conducted after colonies had fasted for 2 d.

Effect of High Temperature on Ant Behavioral Response Gaster secretions were obtained by excising the gasters of 20 chilled ants and then extracting their compounds over 24 h by using 400 μl hexane. This extract then was diluted to obtain the different concentrations (1:1, 1:10, 1:100 v:v extract:hexane). Experiments were conducted every day with fresh extracts.

For both species, a trail of 10 μl of gaster extract was manually deposited on a 2 cm wide, 25 cm long test glass bridge (bridge X, hereafter) using a needle; 10 μl of pure hexane were laid on a control glass bridge (bridge C, hereafter). Bridges X and C were maintained for 10 min at 25, 35, 45, or 55 °C. Both bridges then were cooled to room temperature (25 °C) for 5 min. In order to test whether the ants were able to choose the previously marked bridge, a Y-shaped device was set-up with bridges X and C as the diverging branches; neither bridge lead to food. The location of both bridges (either left or right arm of the Y) was randomly chosen before each trial. Following the connection of the bridges to their foraging area, the number of ants crossing each branch was counted for 2 min. Five replicates were conducted per colony ($N=6$ colonies per species), temperature, and extract concentration.

The probability of an ant choosing bridge X was analyzed by fitting a GLZ. The dependent variable was the number of ants crossing bridge X (success) and C (failure). Predictor variables were the species (2-level categorical variable), the treatment temperature (4-level categorical variable), and the dilution level of the extract (3-level categorical variable). A full GLZ including all effects and interactions was fitted using the quasi-binomial family. Non-significant interactions were progressively removed in accordance with *F*-test scaled deviances (see Supplemental Material 1 for model selection). Statistical analyses were performed using the R software (R Development Core Team, 2010).

Abdominal Gland Secretions

Gaster Extract Composition The abdomens of 10 to 20 freshly freezer-killed *T. nigerrimum* workers were placed in 1 ml of hexane for 24 h to extract the relevant compounds. The extract was concentrated to 100 μl under nitrogen flow and 2 μl were injected into a Perkin-Elmer GC-MS operating at 70EV with a DB-5 fused silica capillary column (length 30 m, inner diam 0.25 mm, and film thickness 0.10 μm). Oven temperature was 1) held at 50 °C for 5 min, 2) raised to 150 °C at a rate of 5 °C/min 3), then raised to 320 °C at rate of 15 °C/min, and 4) held at 320 °C for the last 5 min. This program enabled us to separate mostly volatile compounds [presumably originating from the pygidial gland as in *T. simrothi* as described by Simon and Hefetz (1991)] from mostly non-volatile cuticular hydrocarbons. Twelve replicates were analyzed to obtain the relative chemical composition of the gaster extracts. Compound quantification was achieved by adding 400 ng of Eicosane as an internal standard to the extracts before running the GC-MS.

The trail pheromone of *Aphaenogaster senilis* is composed of a mixture of secretions from the Dufour and poison glands (Lenoir et al., 2011). The former mostly contains hydrocarbons (Boulay et al., 2007a), while the latter contains significant amounts of alkaloids (Lenoir et al., 2011). We verified that the profiles of our experimental colonies were identical to those previously described for this species.

Compound Volatility Initial gaster exudate solutions were extracted from 150 gasters excised from chilled ants and placed in 1 ml hexane for 24 h. A first chromatography run was performed using 20 μl of the initial extract diluted with 1.4 ml dichloromethane (“extract” solution). To obtain the “control” solution, 20 μl of the initial solution were deposited on a glass slide with a needle; the glass slide was immediately washed with 1.4 ml dichloromethane, and the retrieved solution was analyzed in GC-MS. The aim of the "extract" and "control" solutions was to identify the compounds too volatile to be recovered after deposition on the glass slide. With the temperature-treatment solutions, 20 μl of the initial solution were deposited on a glass slide with a needle; the glass slide was maintained at 25, 35, 45, or 55 °C for 10 min before being washed with 1.4 ml dichloromethane and analyzed in GC-MS.

Before analytical chromatography was performed, 5 μl of C24 (375.10^{-5} mg) were added as an internal standard. Compounds were separated using a gas-chromatograph (GC-2010 *Shimadzu*) with a DB-5HT fused silica capillary column (length 30 m, inner diam 0.25 mm, and film thickness 0.10 μ*m*). The temperature 1) was set at 50 °C for the first 2 min, 2) raised to 200 °C at a rate of 10 °C/min, 3) increased to 300 °C at rate of 20 °C/min, and held at 300 °C for the last 5 min.

Only compounds for which the concentration was higher than 10 ng in 1.4 ml of the extract solution were used in statistical analyses. Removed small peaks represent 12.69 % (sd=1.92) for *A. senilis*, and 10.87 % (sd=5.75) for *T. nigerrimum* of the total amount of chemicals. For both species, compounds were regrouped into 4 categories according to their retention time. First, a Friedman rank sum test was performed to compare the total amount of compounds (irrespective of the category) in the initial extract and in the 5 retrieved solutions (control, 25, 35, 45, and 55 °C). Then, if any significant differences were detected, Kruskal-Wallis rank sum tests were performed on each category of compounds. The same procedure was repeated for all temperature-treatment solutions (25, 35, 45, and 55 °C).

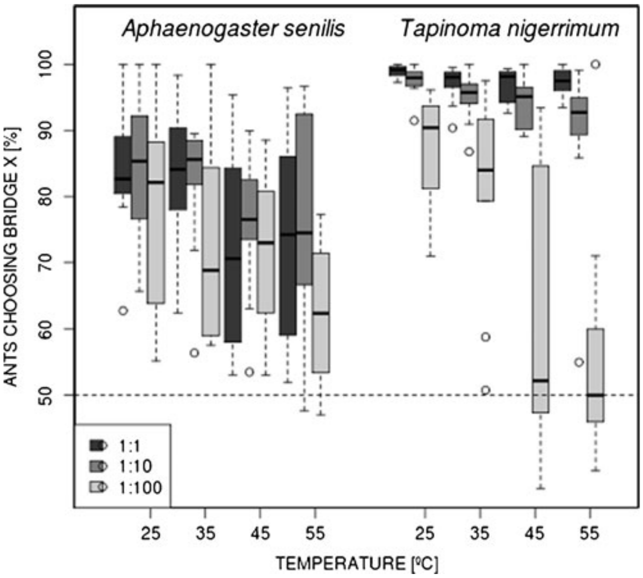

Fig. 1 Box plots of the proportion of *Aphaenogaster senilis* and *Tapinoma nigerrimum* workers choosing the chemically marked bridge (X) based on the temperature treatment and the dilution level of the gaster extract (1:1, 1:10, and 1:100, see insert). For each species, extract dilution, and temperature treatment, $n=10$. The *box* represents ±25 % of the data, the *bold line* marks the median value, and *vertical dot lines* stand for the acceptable range (±1.5*interquartile distance)

Results

Effect of High Temperature on Ant Behavioral Response At 25 °C, significantly more workers of both species chose bridge X (previously marked) than bridge C (control) ($t_{216}=4.3$, $P<0.001$ for *A. senilis* and $t_{216}=8.0$, $P<0.001$ for *T. nigerrimum*). The choice was more consistent over trials with *T. nigerrimum* than with *A. senilis* (Fig. 1). In both species, ants were less likely to choose bridge X at higher temperatures ($F_{3,\,236}=16.8$, $P<0.001$; Fig. 2). However, at low extract concentrations, *T. nigerrimum* was more affected by temperature increases than *A. senilis* (Fig. 2). The dilution level of the gaster extract was essential to predicting the probability of ants choosing bridge X. The interaction between dilution and temperature effects was significant and different between the two species (Fig. 2). In *T. nigerrimum*, workers' choosiness significantly decreased with pheromone exudate dilution. At the highest extract concentration (1:1), the temperature treatment had no effect on ant choice. At the lowest extract concentration (1:100), ants preferentially chose the marked bridge after mild temperature treatments but failed to discriminate between bridges after high temperature treatment (Fig. 2). Although *A. senilis* was not sensitive to differences in more

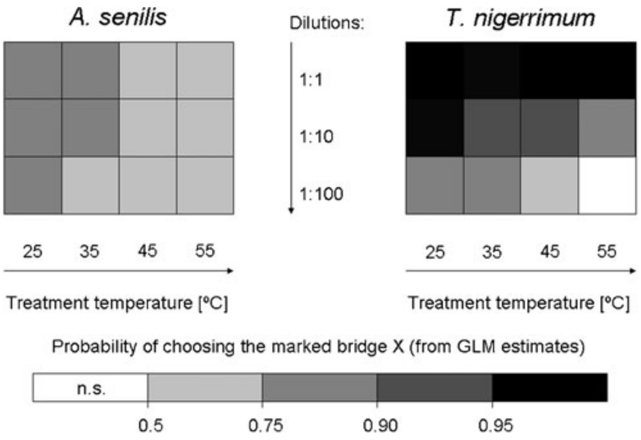

Fig. 2 Probability of choosing the marked bridge X by workers of each ant species during experiments. Trail-following probability was estimated from the GLZ as a function of the species, the dilution level of the gaster extract, and the temperature treatment. The GLZ maximum-likelihood estimates were transformed with logistic function in order to represent the estimated probability of choosing the previously marked bridge X for the two species *Aphaenogaster senilis* and *Tapinoma nigerrimum*

concentrated dilutions (1:1 vs. 1:10, Fig. 2), ant choice was significantly biased when exposed to greater dilution differences (1:10 vs. 1:100, Fig. 2).

Abdominal Gland Secretions

Gaster Extract Composition Gaster compounds extracted from *T. nigerrimum* were dominated by significant amounts of monoterpene iridodial isomers (63.05 %±8.09; Fig. 3), ketones (2-methyl-4-heptanone, 6-methyl-5-hepten-2-one (sulcatone), 4-nonanone, and tridecanone, 29.38 %±8.27), and iridomyrmecins (7.16 %±4.05). Some alkanes, alkenes, and alcohols also were present in small quantities. The average quantity of each compound per individual was variable most likely due to worker polymorphism. There was about 116.7±19.1 ng of light ketones, 259.7±56.3 ng of iridodials, and 21.5±5.4 ng of iridomyrmecins per individual.

We confirmed that the chemical profile of *A. senilis* abdominal glands was identical to that previously obtained for this species (Lenoir et al., 2011). The abdominal gland secretions of *A. senilis* were mostly composed of hydrocarbons and copious amounts of alkaloids.

Compound Volatility To study their stability at high temperatures, main compounds were grouped according to their retention times. For *T. nigerrimum,* the first group consisted of the light ketones (2-methyl-4-heptanone, 6-methyl-5-hepten-2-one (sulcatone), 4-nonanone): they were highly volatile since they did not persist once deposited (presence in the extract solution and absence in the control solution, Fig. 4b). Iridomyrmecins formed the second group. The third group contained 2-tridecanone. Both Iridomyrmecins and 2-tridecanone were of short durability since they persisted once laid, but dissipated in less than 10 min (presence in the control solution and absence in the 25 °C treatment, Fig. 4b). The fourth group was composed of stable aldehydes, the iridodials. Their quantity did not vary across all treatments (Table 1).

Regarding *A. senilis* gaster extract*,* we confirmed that the chemical profile of *A. senilis* abdominal glands was identical to that previously obtained for this species (Lenoir et al., 2011). The abdominal gland secretions of *A. senilis* were composed mostly of hydrocarbons and copious amounts of alkaloids. The main compounds were grouped according to their retention times and volatility. First, tridecane (C13) was found in small concentrations (<250 ng per sample), rapidly vanished as temperature increased, and could not be detected after the bridge was heated to 55 °C (Table 2, Fig. 4a). Second, pentadecene and pentadecane coeluted with two alakaloids pyrazine and anabaseine. Both alkanes originated from the Dufour gland, while the alkaloids originated from the poison gland. Their concentration decreased rapidly with increasing temperature (Fig. 4a). They disappeared completely from 35 °C onwards (Fig. 4a). Third, three C17 alkanes and alkenes showed the same qualitative pattern as the second group, but their initial quantity was lower and they totally disappeared at 55 °C (Fig. 4a). The fourth group was stable and the quantity of compounds did not change between the different treatments (Table 2). It was formed by a mixture of nonadecene and nonadecane.

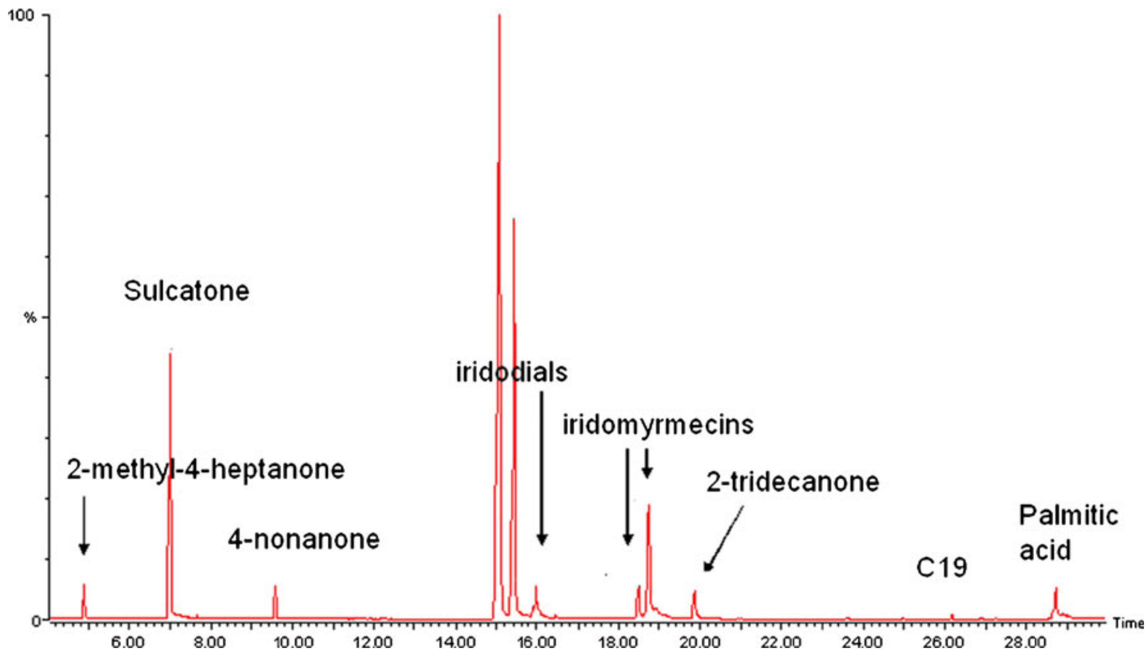

Fig. 3 Chromatogram of *Tapinoma nigerrimum* worker gaster extract obtained from GC-MS. The most abundant compounds are indicated

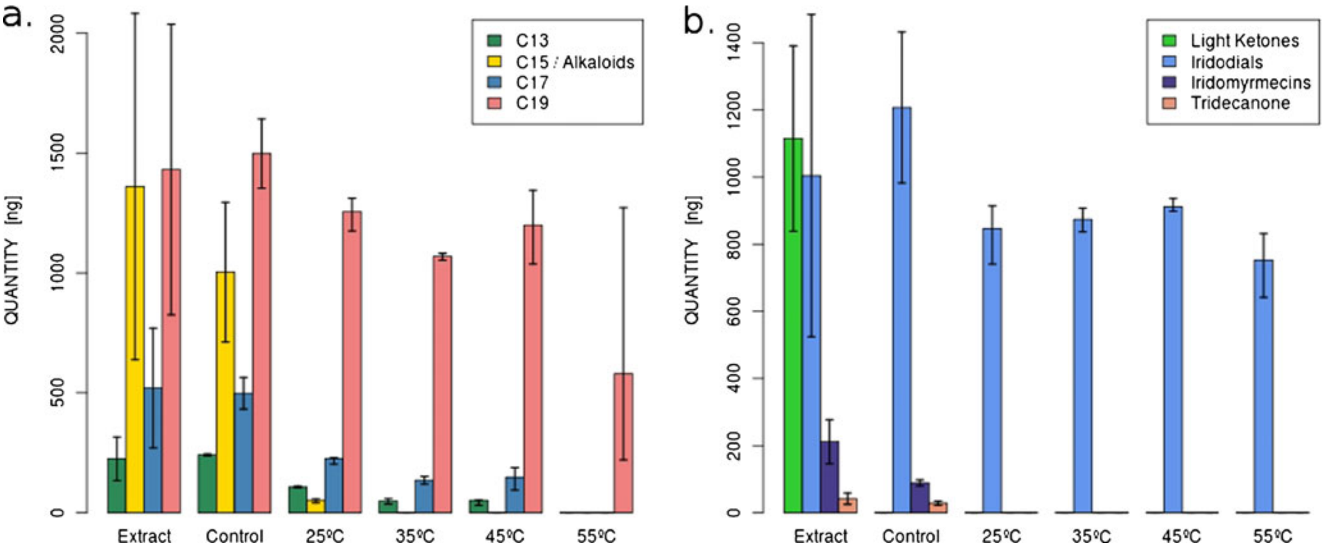

Fig. 4 Variation in (**a**) *Aphaenogaster senilis* and (**b**) *Tapinoma nigerrimum* worker gaster exudate composition across the different experimental treatments. The different solutions were analyzed by gas-chromatography using an initial concentration of 3 gasters

Discussion

High temperature differently affected the foraging behavior of *Aphaenogaster senilis* and *Tapinoma nigerrimum*. Trail-following experiments demonstrated that exposing gaster extract to increasing temperatures affected *T. nigerrimum* worker behavior more than *A. senilis*. Chemical analyses determined the composition of the gaster extract of both species and discriminated between the volatility of their different compounds. Linking chemical analyses and behavioral experiments allows one to identify the essential compounds for trail following behavior in both species.

The analysis of *T. nigerrimum* extract composition revealed the presence of remarkably stable compounds: the iridodials. These compounds also are involved in the recruitment process of a closely related species, *Tapinoma simrothi,* and have a half-life of 11 days (Simon and Hefetz, 1991). The strong persistence of this pheromone and an excellent trail following behavior (>90 % of workers, Fig. 1) might be advantageous in the exploitation of stable food sources such as aphid-honeydew (Cerdá et al., 1989).

Table 1 Volatility of the compounds involved in *Tapinoma nigerrimum* worker gaster exudate composition. "All solutions" tests identify absolute differences in concentration between the initial extract solution (initial concentration equivalent to 3 gasters), the control solution (laid and immediately retrieved from a glass bridge), and temperature treatments (10 min. at 25, 35, 45, or 55 °C on a glass bridge). "Temperature treatments" test identifies differences in concentration exclusively due to temperature raising. Nonparametric tests were performed on the quantity of the compounds established by gas-chromatography

Discriminating effect	Test	Df	χ_2	*P*-value
All solutions				
All compounds	Friedman	15	36.9	0.001
Ketones	Kruskal-Wallis	5	14.9	0.011
Iridodials	Kruskal-Wallis	5	7.0	0.220
Iridomyrmecins	Kruskal-Wallis	5	14.9	0.011
Tridecanone	Kruskal-Wallis	5	14.7	0.012
Temperature treatments				
All compounds	Friedman	11	11	0.443

Table 2 Volatility of the compounds involved in *Aphaenogaster senilis* worker gaster exudate composition. "All solutions" tests identify absolute differences in concentration between the initial extract solution (initial concentration equivalent to 3 gasters), the control solution (laid and immediately retrieved from a glass bridge), and temperature treatments (10 min. at 25, 35, 45, or 55 °C on a glass bridge). "Temperature treatments" tests identify differences in concentration exclusively due to temperature raising. Nonparametric tests were performed on the quantity of the compounds established by gas-chromatography

Discriminating effect	Test	Df	χ_2	*P*-value
All solutions				
All compounds	Friedman	15	51.2	<0.001
C13	Kruskal-Wallis	5	13.9	0.016
C15/alkaloids	Kruskal-Wallis	5	14.6	0.012
C17	Kruskal-Wallis	5	14.0	0.016
C19	Kruskal-Wallis	5	6.8	0.233
Temperature treatments				
All compounds	Friedman	11	35.1	<0.001
C13	Kruskal-Wallis	3	9.5	0.023
C15/alkaloids	Kruskal-Wallis	3	10.7	0.013
C17	Kruskal-Wallis	3	9.6	0.022
C19	Kruskal-Wallis	3	4.1	0.248

Notwithstanding, despite this compound's stability, the ants' detection of the gaster extract failed under disadvantageous conditions (low gaster extract concentration and temperature treatments >35 °C). Indeed, to induce optimal trail-following, traces of the more volatile compounds might be necessary, as in many other ant species (Morgan, 2009). To efficiently forage at high temperatures, the trail might, thus, necessitate constant reinforcement. In order to do so, foragers would risk exposure to higher temperatures. Consequently, the strong limitation placed by temperature on the foraging schedule of *T. nigerrimum* might depend on the interplay between the physiological limitations of foragers and pheromone decay. *Aphaenogaster senilis* does not rely as strongly on chemical communication as *T. nigerrimum*. Chemical analysis of gaster extracts revealed the importance of C19 hydrocarbons in the trail-following behavior of *A. senilis*. However, increasing gaster extract concentration did not necessarily improve trail-following, and workers demonstrated important behavioral variability (Fig. 1). This species' foraging efficiency might, thus, rely on other kinds of cues such as physical contact with the leader (Hölldobler, 1971) and individual skills like load size (Cerdá et al., 1998b) or orientation ability, as in individual foraging species (Collett et al., 1992).

As far as the model species are representative, their mode of recruitment illustrates two different strategies. Mass recruiters like *T. nigerrimum* is based on an investment in chemical communication, allowing the rapid recruitment of numerous nestmates, and thereby the control of long-lasting food sources. However, variations in environmental conditions that affect pheromone concentration, such as hot ground temperatures, interfere with the recruitment process, and disrupt both the foraging and the competitive abilities (Cerdá et al., 1997). Group recruitment, on the other hand, allows the collective retrieval of short-lasting resources such as dead insects (Cerdá et al., 2009), but does not enhance competitive superiority. Notwithstanding, since foragers are not restricted by chemical cues, fluctuating environmental conditions do not affect their foraging activity.

At the community level, species coexistence might be promoted by these different investments in strength vs. flexibility, or competitive ability vs. abiotic tolerance. In Mediterranean-like communities, dominant species are able to displace subordinates but are less tolerant to high temperatures. This restriction is partly due to physiological limitations (e.g., the Critical Thermal Limit of *T. nigerrimum* is 4 °C lower than that of *A. senilis*), but also to behavioral differences regarding foraging activity. However, this "dominant/subordinate" terminology might be somehow misleading: it contributes to the idea that subordinate species are maintained in the community by exploiting suboptimal niches. On the contrary, subordinates have an ecological niche with extended dimensions that allows them to face changing environmental conditions without losing efficiency. Their strategy is, thus, fully adapted to fluctuating environments like terrestrial Mediterranean ecosystems.

We focused on the effect of high temperatures on trail-following behavior according to the kind of recruitment used by different species. In our model system, ants' trail-following was more altered by increasing temperature in the mass-recruiting species than in the group-recruiting species. This result suggests a functional mechanism for the Dominance-Thermal tolerance trade-off in semi-arid ant communities (Cerdá et al., 1998a; Wittman et al., 2010): since behavioral dominance relies on a species' ability to recruit nestmates, high temperatures, by reducing recruitment efficiency, might restrict dominance ability at the community level. Trade-offs between competitive ability and stress tolerance are common in many different communities (e.g., marine communities, plant communities) and it would be worth assessing the functional mechanism of such trade-offs in other systems to gain a better understanding of community diversity.

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