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MOLECULAR SIGNALS AND RECEPTORS: CONTROLLING RHIZOSPHERE INTERACTIONS BETWEEN PLANTS AND OTHER ORGANISMS

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Abstract. Rhizosphere interactions are affected by many different regulatory signals. As yet, however, only a few have been identified. Signals, by definition, contain information, react with a receptor, and elicit a response. Signals may thus represent the highest level of evolved response in rhizosphere communities and, in that sense, occupy a supreme control point. At the same time, some signals may function as modulators of downstream responses, rather than on/off switches. To assess these possibilities, several interactions between plants and soil organisms are described, starting with the molecular interactions between leguminous plants and symbiotic bacteria of the family Rhizobiaceae, one of the best-characterized plant–microbe associations in the rhizosphere. We then examine other interactions between plants and soil organisms for overlap and/or connections with the rhizosphere signals utilized in the legume–*Rhizobium* symbiosis. Whether information currently available reflects the interaction of the organisms in nature or only in the laboratory has not always been determined. Thus, the key ecological issue of how important some of the signals are under field conditions remains to be addressed. Molecular tools now available make this task less daunting than in the past, and thus a new age of experimental field ecology may soon burst forth in rhizosphere studies. By identifying the signals, receptors, and the critical control points, we can better understand the organismal dynamics in this key belowground ecosystem.

Key words: parasitism; pathogenesis; predation; quorum sensing; receptors; rhizosphere interactions; signals; symbiosis.

SPECIAL FEATURE

INTRODUCTION

Rhizosphere food webs are highly integrated. They are very much influenced by the organisms and the edaphic factors that are present. Soil-inhabiting mutualists and parasites, both prokaryotic and eukaryotic, are involved in rhizosphere signaling with a host (Fig. 1). The diverse regulatory signals influencing these interactions contain information, which is perceived by receptors and transduced via downstream effectors. In so doing, this leads to mutualist/parasite recognition and associated responses with the host.

Much of the signaling between plants and other organisms is based on plant-derived chemicals, but signals are produced by the interacting organisms as well. Moreover, there is significant overlap in the chemical language used for signaling. For example, molecules that attract pollinators, seed dispersers, and herbivores are structurally related to those that attract or repel subterranean microbial mutualists and pathogens. In the soil, both *Bradyrhizobium japonicum* (mutualist) and *Phytophthora sojae* (pathogen) respond to isoflavones released by soybean roots. Related molecules are used by parasitic plants as cues to the whereabouts of their nearest plant neighbor and potential victim (Fig. 2). How do organisms interpret the myriad signals and use them to trigger specific developmental events? How are redundant and overlapping signals prioritized? How are signal gradients recognized? Whether information currently available reflects how these different organisms interact in nature or only in the laboratory has not been determined in all cases. Thus, the key ecological issue of how important some of the signals are under field conditions remains to be addressed.

In this review, interactions between plants and other organisms are examined at two distinct stages: (1) before the encounter between the organisms and (2) upon recognition. In the process, we discuss a diverse group of organisms, albeit focusing in each section on a few organisms. For each topic, analyses of mutualism, pathogenesis/parasitism, and predation demonstrate that shared signals operate. Where possible, we also de-

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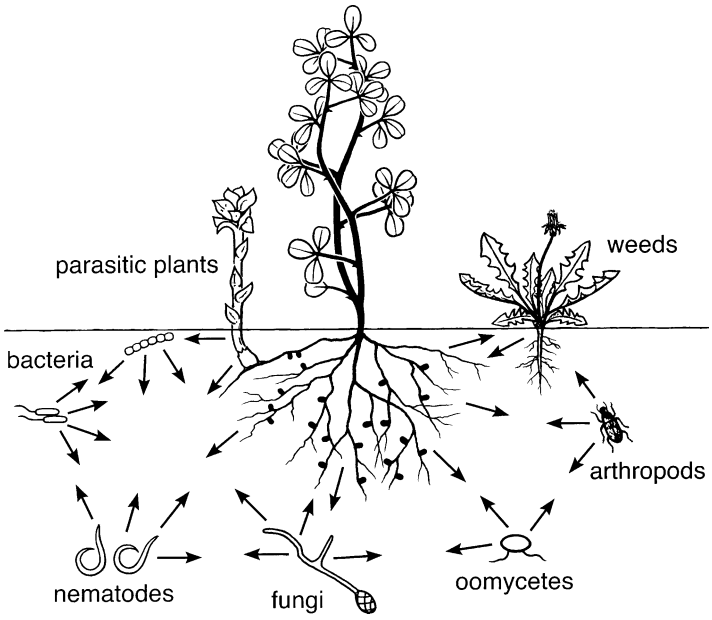

FIG. 1. A representation of the complex interactions that take place underground between plant roots and other organisms. Arrows indicate the molecular signaling that occurs. Organisms are not drawn to scale.

scribe the potential control points—defined as those regulatory elements that are operated on by selection processes to confer fitness on an individual organism, thereby having effects on propagation through other trophic levels (Phillips et al. 2003).

The major questions for this review are the following: (1) what are these signals, (2) with which receptor(s) do they interact, and (3) how do signals regulate the fates of the interacting organisms? Lastly, can we utilize the signals and receptors at these critical control points to monitor the association between plants and rhizosphere organisms in the field?

CHEMICAL SIGNALING BEFORE THE ENCOUNTER

Chemotaxis and gene induction

Soil contains a suspension of particles composed of inorganic and organic material dispersed in an aqueous environment. Here, diffusion is constrained, with chemical signals most likely being active for distances of only a few microns to millimeters from the extending roots. Signal molecules frequently exchanged between organisms in the rhizosphere include the phenolics, aromatic metabolites derived from the phenylpropanoid biosynthesis pathway. Phenolics, particularly those polymerized as lignin, account for ~40% of the organic carbon in the biosphere, reflecting their central roles in plant development.

Plants have repeatedly recruited phenolics as signal molecules, either to facilitate or to discourage interactions with other organisms. The colors of fruits and flowers, and subsequently their notice by insects and animals, are frequently functions of phenolic composition. Symbiotic microbes such as rhizobia are attracted to, and their *nod* genes are induced by, phenolics (reviewed in Schultze and Kondorosi 1998). In contrast, ingress of pathogenic microbes can be curtailed in incompatible interactions by complex phenolics known as phytoalexins (reviewed in Dixon and Paiva 1995). Plant phenolics similarly act as repellents of multicellular herbivores including insects, birds, and mammals (Jakubas et al. 1989, Palo and Robbins 1991, Schultz et al. 1992). Furthermore, phenolics are the most common class of allelopathic compounds, molecules released by one plant that affect the growth or development, either positively or negatively, of a second (Inderjit 1996).

The signaling potential of flavonoid aglycones, phenolic compounds produced exclusively by plants, is particularly well documented. More than 4000 different flavonoids have been identified in vascular plants (Harborne 1988). All flavonoids have a common diphenylpyran skeleton—two benzene rings linked through a heterocyclic pyran or pyrone ring. This basic ring structure allows a number of substitutions in the C-ring that give rise to flavonols, flavones, flavanones, anthocyanidins, and isoflavonoids. Many are esterified at hydroxyl groups with different sugars; commonly glucose, galactose, rhamnose, and rutinose; to produce glycosides. Flavonoids range from those that are very lipophilic to those that are water soluble. The size of monomers also varies from the MW 208 of chalcone to the MW 1759 of heavenly blue anthocyanin.

The structural diversity represented within the flavonoids is further augmented through transformations between different electrochemical states. Cheminat and Brouillard (1986) identified nine potential electrochemical conformations of anthocyanins in water. The conformation of the dominant flavonoid species at equilibrium in the rhizosphere will be a function of multiple factors including pH, water potential, and anion avail-

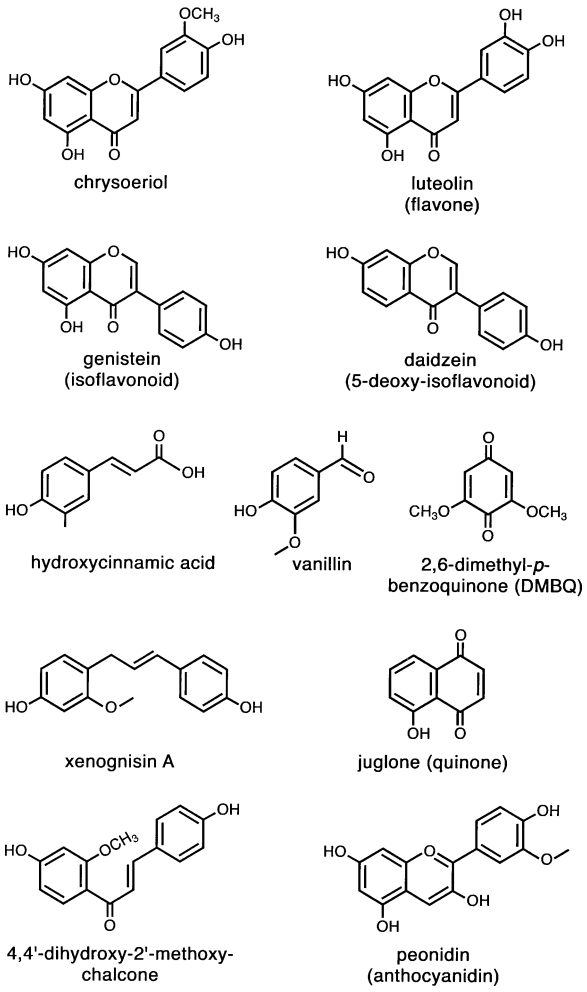

FIG. 2. Some phenolics utilized in signaling between plant roots and other organisms in the soil. See *Chemical signaling before the encounter* for details.

ability. In many cases, the bioactivity of a phenolic molecule is a function of its redox state (Appel 1993). For example, the phytotoxicity of juglone (Fig. 2), a compound released by walnut trees that inhibits the growth of neighboring plants, depends on its redox state (Lee and Campbell 1969). As discussed later, the identification of host recognition molecules by parasitic Scrophulariaceae is a function of redox cycling between phenolic states (Smith et al. 1996). There is also a large body of evidence that the effectiveness of certain phenolics and quinones (oxidized phenolics) as pharmacological agents against fungi, malaria, and cancer is primarily redox related (O'Brien 1991). Redox transformations of rhizosphere phenolics further enhance their information carrying potential.

Rhizobia

Flavonoid profiles differ considerably among legumes. This specificity enables mutualists such as rhizobia to distinguish their hosts from other legumes. How this is accomplished is relatively well known, at least based on laboratory studies.

NodD and SyrM proteins are positive transcriptional activators of rhizobial *nod* genes that have a *nod* box in their promoters. They are members of the LysR family of transcriptional activators that have a helix-turn-helix DNA binding motif in the N terminus of the protein (reviewed in Schell 1993). Some rhizobia have only one *nodD* gene whereas *Sinorhizobium meliloti*, the symbiont of *Medicago*, *Melilotus*, and *Trigonella*, has three and also *syrM*, a closely related gene that encodes an activator, which controls both *nod* gene expression and exopolysaccharide synthesis. The *nodD1* gene product of *S. meliloti* interacts strongly with luteolin, chrysoeriol, 4,4′-dihydroxy-2′-methoxychalcone (Fig. 2), or 7, 4′-dihydroxyflavone, but can also interact with other flavonoids. Some simple phenolics such as hydroxycinnamic acids and vanillin (Fig. 2) also induce rhizobial *nod* genes (Le Strange et al. 1990, Kape et al. 1991). NodD2 utilizes betaines as coinducers, whereas neither NodD3 nor SyrM require a plant-derived molecule (reviewed in Phillips 2000). In *S. meliloti*, NodD and SyrM form a complex autoregulatory network (reviewed in Schlaman et al. 1998). Transcription of *syrM* is activated by *nodD3* and vice versa. Once induced, the products of the *nod* genes direct the synthesis of a molecule called Nod factor (Fig. 3A), a lipochitooligosaccharide (LCO) bacterial signal molecule that enables the rhizobia to establish N_2-fixing nodules on roots of their host legume. Proteomic studies have shown that the flavone luteolin affects the levels of many proteins in *S. meliloti* (Chen et al. 2000). This finding greatly expands the potential signal functions of plant flavonoids in interactions with associated bacteria.

Various *nodC–lacZ* fusions have been used to monitor expression of *nod* genes in response to plant-produced compounds (Innes et al. 1985, Mulligan and Long 1985, Rossen et al. 1985). A practical application of the *nodC–lacZ* fusions for ecological studies is as a biosensor to monitor flavonoids or other molecules (e.g., endocrine-disrupting chemicals, Fox et al. 2001) either produced by plant tissues or material found in the soil. Studies extracting *nod*-gene inducing activity from the alfalfa rhizosphere demonstrated that 7,4′-dihydroxyflavone and 7,4′-dihydroxyflavanone, compounds previously identified in the laboratory as *nod*-gene inducers in root extracts, are present in the soil (Léon-Barrios et al. 1993, Phillips et al. 1997). Availability of biosensors should facilitate such field-based analyses.

Another approach, which could be adapted to field studies, used *S. meliloti* cells containing *gfp* (green fluorescent protein) fused to the *melA* promoter to monitor root colonization (Bringhurst et al. 2001). The *melA* gene is induced upon exposure to galactose and galactosides. The rhizobia exhibited bright fluorescence, not only in the presence of alfalfa roots (their

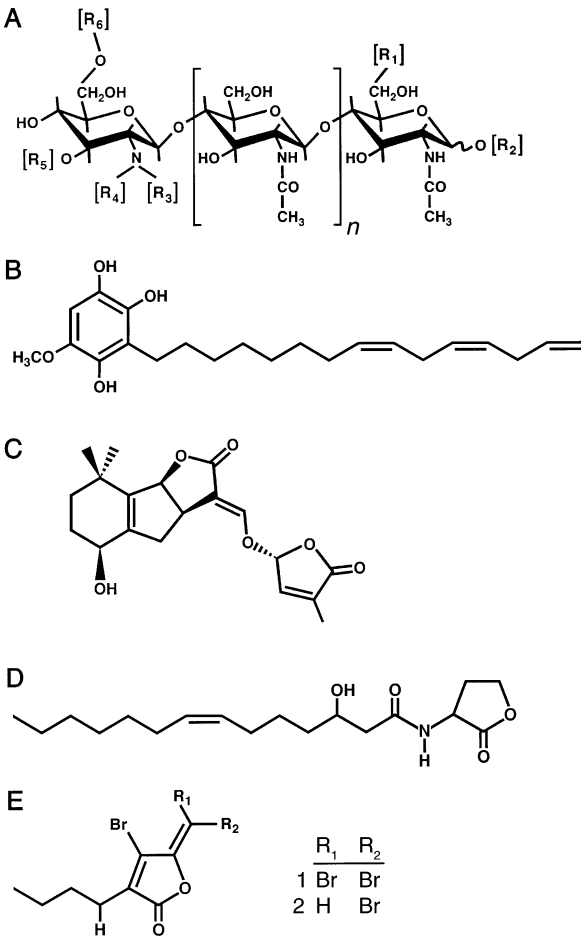

FIG. 3. Some nonphenolic signal molecules. (A) Generalized structure of Nod factor, which is derived from the expression of the rhizobial *nod* genes. The number of glucosamines in the chitin-like backbone (*n*) typically is four or five. Depending on the rhizobial strain, R_1 can be H, sulfate, fucose, methylfucose, sulfomethylfucose, acetylmethyfucose, or D-arabinose. R_2 can be H or glycerol whereas R_3 is either H or CH_3. R_4 is a C-16 or C-18 fatty acid and R_5 can be either H or a carbamoyl group. R_6 can be H, or an acetyl or carbamoyl group. (B) SXSg; sorghum xenognosin of *Striga* germination, produced by sorghum roots. An active but highly unstable molecule. (C) Strigol, a sesquiterpene, was the first parasitic plant seed germination stimulant identified. (D) AHL (*N*-acylated homoserine lactone) from *Rhizobium leguminosarum* bv. *viciae*. (E) Two furanones produced by the red alga *Delisea pulchra*.

normal host), but also when inoculated onto monocot roots. This type of reporter gene construct thus monitors root colonization and presumably galactose utilization. It also suggests, based on expression of *melA*, that the rhizobia accrue a major benefit from an association with roots, both leguminous and nonleguminous.

Parasites

Parasitic plants obtain at least some of their nutrients by invading the tissues of other plants (Press and Graves 1995). The degree to which parasitic Scrophulariaceae rely on host resources varies from facultative parasites that reach maturity in the absence of host plants, but will attach to and invade a variety of monocot and dicot roots when available, through obligate parasites that have lost photosynthetic activity and require host resources almost immediately after germination. The effects of plant parasitism on host plants can be debilitating. Some of the world's most pernicious agricultural pests are parasitic weeds (Parker and Riches 1993).

Striga and *Orobanche*, two of the most devastating parasitic weeds, require specific host factors to break seed dormancy, thereby ensuring the immediate availability of a host root. It is interesting that these two parasites recognize similarly structured germination factors in light of the phylogenetic evidence that obligate heterotrophy arose independently during the evolution of these genera (Nickrent et al. 1998). The most common germination stimulants are sesquiterpene lactones, collectively referred to as strigolactones, including: strigol, the first germination stimulant identified (Fig. 3C; Cook et al. 1966), sorgolactone, orobanchol, and alectrol (Wigchert and Zwanenburg 1999). Strigolactones stimulate germination at low concentrations (1×10^{-8} to 1×10^{-12} mol/L) and structural studies of these and synthetic analogs show that bioactivity resides in the CD rings with their absolute configuration being critical (Thuring et al. 1997). Together these results suggest that *Striga* perceives these molecules through a receptor-mediated process (Mangus and Zwanenburg 1992).

Another class of germination factor identified in host exudates is the dihydrosorgoleone referred to SXSg (sorghum xenognosin of *Striga g*ermination; Chang et al. 1986; Fig. 3B). The active hydroquinone of SXSg is rapidly oxidized to inactive sorgoleone, demonstrating that the bioactivity of SXSg is dependent on its redox state (Fate et al. 1990). Oxidative inactivation of SXSg may explain why *Striga* seeds need to be close to host roots in order to germinate (Chang et al. 1986). The concentration of SXSg needed for half maximal germination of *Striga* seed is at least three orders of magnitude higher than that of strigolactone, so it is likely that these two classes of germination factors are perceived differently (Wigchert and Zwanenburg 1999).

After germination, a second developmental process, haustorium development, is triggered in parasitic Scrophulariaceae in response to host-root signal molecules. The haustorium is a globular-shaped root structure that attaches the parasite to the host root, invades the host tissue, and establishes a vascular continuity through which the parasite translocates host resources (Press and Graves 1995). Parasitic Scrophulariaceae typically do not develop haustoria unless grown in the presence of host roots. The application of host root exudates to in vitro-grown parasitic seedlings established that the

haustoria-inducing factor(s) was released from the root (Riopel and Musselman 1979). The first factors purified were the flavonoids xenognosin A (Fig. 2) and B (Lynn et al. 1981, Steffens et al. 1982). In vitro assays later identified a redundancy in haustorium-inducing factors that included simple phenolic acids, quinones, and anthocyanidins (Fig. 2; Chang and Lynn 1986, Riopel and Timko 1995, Albrecht et al. 1999).

A mechanistic model to account for the haustorial-inducing activity of diverse phenolics suggests that the core inducing molecule is the unstable, reactive semiquinone intermediate formed during redox cycling between quinone and hydroquinone forms (Keyes et al. 2000). Although there are certain structural requirements for active vs. inactive haustorial inducers, the most striking feature of active molecules is that their electrical potentials fall within a relatively narrow redox window (Smith et al. 1996). The inhibition of haustoria initiation by certain chemical spin traps substantiated the importance of the radical intermediate in haustorium signaling (Zeng et al. 1996).

Pathogens

Phytophthora sojae is an oomycete pathogen that infects soybean roots. Oomycetes are physiologically and morphologically similar to fungi, but phylogenetically distinct, being more closely related to diatoms and brown algae. Chemical recognition events in the rhizosphere constitute the earliest steps in the *Phytophthora*-soybean interaction, as they do in other pathogen–root interactions.

The zoospores and hyphae of *P. sojae* recognize nanomolar concentrations of daidzein and genistein (Fig. 2; Morris and Ward 1992, Tyler et al. 1996, Morris et al. 1998). These soybean signals stimulate chemotaxis by the motile zoospores, encystment of the zoospores, germination of the cysts, and chemotropism by hyphae emerging from germinating cysts. Although *P. sojae* zoospores are sensitive to nanomolar concentrations of the isoflavones, they also respond to a wide range of phenolic compounds in the 1×10^{-7} to 1×10^{-5} mol/L range, including flavones, stilbenes, chalcones, coumarins, and chromanones, suggesting that the organism may in fact integrate a large amount of information about its chemical environment. The nature of the isoflavone receptor is currently unknown. Genetic analysis of natural variation in chemotaxis preferences has identified a single locus that controls isoflavone detection, but a much larger number of segregating loci probably control responses to phenolic compounds other than isoflavones (J. Wang and B. Tyler, *personal observations*).

Nematodes

Given the size of the phylum ($\leq 1 \times 10^8$ species; Boucher and Lambshead 1994), it is perhaps not surprising that parasitic nematodes have evolved to exploit all parts of vascular plants (Bird and Koltai 2000). As is the case for the mutualistic and parasitic symbionts described above, the sedentary, endoparasitic cyst and root-knot nematodes (*Heterodera*/*Globodera* and *Meloidogyne* spp., respectively) exhibit complex and intimate associations with their host plant. There is little doubt that such associations involve reciprocal signaling between host and parasite, but unlike rhizobia, parasitic plants, and *P. sojae*, such signaling can only be inferred. With a few exceptions, the nature of the signaling molecules remains unknown. Nevertheless, the case for the central role of signaling molecules in the host–parasite interaction is strong, and it is consistent with data obtained for the free-living nematode *Caenorhabditis elegans*. Parasitic nematodes have a well-developed nervous system that includes chemo-, thermo-, and mechanosensory neurons (Bird and Bird 1991), and like *C. elegans*, presumably integrate a wide variety of external stimuli to achieve a rich behavioral repertoire (Bargmann and Mori 1997). Analysis of the *C. elegans* genome revealed that the largest gene class (~1000 members) encodes G-coupled receptors, most of which are believed to be chemoreceptors (Bargmann 1998). This finding underscores the importance of chemical signaling to nematode ecology. Depending on the plant-parasitic nematode species, host signals probably are required for hatching, host location and selection, intra-host migration, feeding-site selection, and sex determination. Additional signals flow to the host (e.g., for feeding-site induction), and between nematodes, including sex pheromones.

Plant-parasitic nematodes exhibit behavioral and developmental adaptations for host location. Root-knot nematode females lay eggs into an egg sac on the surface of the root from which she is feeding, and larvae hatch within days as developmentally-arrested, dauer (J2) larvae. Although J2 larvae persist and migrate in the soil for months, surviving on stored lipid reserves, it is likely that the hatchlings re-infect the same plant as their mother, obviating the need for long-range host location. However, on agar, J2 larvae are specifically and actively attracted over cm distances to germinating seeds (Riddle and Bird 1985). The nature of the chemoattractant remains unknown, but appears not to include the inorganic salts that attract *C. elegans* (Riddle and Bird 1985). In contrast to root-knot nematodes, most of the cyst nematode eggs and the developing nematodes they contain enter a state of dormancy and are packaged into the tanned body of the mother to form the cyst. Thus, diapaused, specific environmental signals are required for the larvae to hatch, and in the absence of the appropriate signals, dormancy can be maintained for many years. For most cyst nematode species, the key recovery cue is a diffusible signal(s) from the host root. Species with a wide host range (e.g., sugar-beet cyst nematode, *H. schachtii*) respond to root diffusates from a wide range of hosts, whereas species with a restricted host range (e.g., potato cyst nematode, *G. rostochiensis*) hatch only when presented with a

signal from potato, tomato, and a few related hosts (Jones et al. 1998). The first hatching factor purified was glycinoeclepin A (Masamune et al. 1982), which is active at 1×10^{-8} mol/L. Extensive chemical analyses of hatching factors have revealed a range of other highly active terpenoids (Jones et al. 1998). Other environmental factors play a role in modulating hatching, and it is clear that the developmental strategy of diapause permits an exquisite adaptation of the nematode to specific host and rhizosphere conditions.

Quorum sensing and mimicry

Diverse bacteria use either *N*-acyl homoserine lactones (AHLs; Fig. 3C), a furanosyl borate ester (AI2), peptides, or γ-butyrolactones as intercellular signal molecules to coordinate behaviors of individual cells in a local population (Fuqua et al. 2001, Whitehead et al. 2001, Chen et al. 2002). Such regulation is known as ''quorum sensing'' (QS) because changes in behavior require a high local concentration of signal-producing bacteria, i.e., a quorum, to accumulate enough signal to activate specific QS signal receptors in the bacteria, which then regulate the transcription of certain genes—dozens or hundreds of genes in some bacteria. Many behaviors subject to QS regulation contribute to the colonization or infection of eukaryotic hosts (Pierson et al. 1999, Visick and Ruby 1999, de Kievit and Iglewski 2000). A given bacterial species can produce several different QS signal compounds and may ''listen'' to the QS signals of other bacterial species. Rhizobia, like many Gram-negative bacteria, utilize QS (Gray et al. 1996, Lithgow et al. 2000, Blosser-Middleton and Gray 2001, Wisniewski-Dye et al. 2002). Several AHLs has been purified and shown to activate the expression of downstream genes such as the *rhiABC* operon of *R. leguminosarum* bv. *viciae* (Rodelas et al. 1999), and the accumulation of dozens of proteins in *S. meliloti* (M. Teplitski, H. Chen, A. Eberhard, M. Gao, B. Rolfe, and W. Bauer, *personal observations*).

Given the general importance of QS signaling in plant-associated bacteria (Pierson et al. 1999), it is interesting that host plants can interfere with the QS of bacteria (Bauer and Teplitski 2001). Pea plants secrete about a dozen unknown compounds that either stimulate or inhibit AHL-dependent behaviors of bacterial reporter strains designed to respond specifically to exogenous AHLs (Teplitski et al. 2000). The compounds from pea are chemically different from known AHLs, even though they elicit AHL-dependent behaviors in the reporter strains. Thus, the active compounds from pea can be thought of as AHL signal-mimics, i.e., compounds that have the biological effects of AHLs but are chemically different. Plant-produced mimics of the AI2 QS signal have been detected in legumes, rice, and algae (M. Teplitski, M. Gao, and W. Bauer, *personal observations*), indicating that plants have evolved signal-mimic compounds that can disrupt QS regulation in bacteria that use QS signals other than AHLs.

If higher plants such as pea, rice, and tomato can make AHL and AI2 signal-mimic compounds, do these mimic compounds contribute to the control of plant–bacteria interactions in the rhizosphere? Perhaps the best reason for thinking that the production of QS signal mimics by plants may be ecologically important is that such mimics have been shown to control the surface colonization of a marine alga, *Delisea pulchra*, by bacteria in natural ocean waters (Givskov et al. 1996, Kjelleberg et al. 1997). Chemically, the *Delisea* signal-mimic compounds have been identified as halogenated furanones (Fig. 3E). The furanone QS mimics specifically inhibited AHL-mediated gene expression in a variety of bacteria by binding to the AHL receptor proteins and preventing receptor binding to DNA (Manefield et al. 1999, Kjelleberg and Steinberg 2002). When *Delisea* growing in natural marine waters was analyzed, large numbers of AHL-producing Gram-negative species that dominate colonization of biotic and abiotic surfaces were found only at the algal base, where surface concentrations of the furanone signals are lowest (Kjelleberg and Steinberg 2002). Thus, it appears that this alga disrupts the normal colonization of its surface by AHL-producing bacteria.

QS signal mimics may be an important element of control for rhizosphere interactions because they could manipulate the formation of stable communities of helpful bacteria. Bacteria, even mutualists such as rhizobia, form biofilms (N. Fujishige and A. Hirsch, *personal observations*), and in the soil, it is likely that microbes sort themselves out in multi-species, three-dimensional arrays on both biotic and abiotic surfaces to metabolize complex substrates cooperatively (Wolfaardt et al. 1994). Intercellular signals, particularly AHLs, are involved in biofilm formation and in guiding different cells to suitable locations within the biofilm (Davies et al. 1998). Over evolutionary time, we can expect plant signal-mimics in the rhizosphere will help establish beneficial communities of bacteria as often as they disrupt harmful behaviors in potential pathogens. The use of reporter-gene constructs and other types of biosensors can help us discern how these molecules are functioning in the field (Farrar et al. 2003).

Recognition and Response

Mutualists

The interaction between plant flavonoids and rhizobial NodD proteins is the first exchange of information in the legume–*Rhizobium* association and, as discussed earlier, results in the induction of *nod* genes. These genes encode mainly enzymes involved in the synthesis of Nod factors (Fig. 3A). Nod factor molecules are LCOs consisting generally of four or five *N*-acetylglucosamines, ß-1–4 linked, with the terminal non-reducing sugar *N*-acylated with a fatty acid of 16

to 18 carbons. Although all rhizobia produce Nod factors with this same generic structure, different species produce characteristic Nod factors with variation in the type and number of chemical substitutions on the reducing and terminal non-reducing sugars and also in the structure of the fatty acid (Perret et al. 2000, Spaink 2000).

The key role that Nod factors play in establishing the symbiosis is illustrated by the following facts: (1) symbiotic rhizobia, despite their diverse phylogenetic origins, all have *nod* genes and hence establish the interaction with legume roots through Nod factor signaling; (2) bacteria mutated in the key *nod* genes involved in the synthesis of the basic LCO structure (*nodABC*) are unable to nodulate; (3) plant mutants deficient in Nod factor signal transduction are deficient in nodulation (reviewed in Hirsch et al. 2001). Although rhizobial cell-surface components and plant lectins also play a role (Perret et al. 2000, van Rhijn et al. 2001), the Nod factors are essential for the three major processes involved in establishing the symbiosis: nodule organogenesis, infection, and determination of host specificity.

To be effective in signaling, Nod factors must be perceived. A major aim now is to identify the nature of the Nod factor receptor(s), and several approaches have described a number of potential candidates (Cullimore et al. 2001). The detailed phenotypes of several plant mutants suggest that they may be altered in genes involved in Nod factor perception. In *Medicago*, a biochemical approach led to the characterization of high affinity binding sites for Nod factors (Gressent et al. 1999, 2002), and in *Dolichos biflorus*, a novel lectin, with apyrase activity, which binds Nod factors has been characterized (Etzler et al. 1999). How these proteins may be involved in the symbiosis is currently being investigated.

Clearly identification of the symbiotic receptors for Nod factors is essential for understanding the mechanisms of Nod factor signaling between the two symbionts. The amount of the Nod factor signal and corresponding receptors, the structure of the Nod factors, and the selectivity of the receptors are key parameters determining whether the Nod factor activates plant signal transduction pathways. In the laboratory, these parameters can be biochemically determined and experiments performed to see how closely the establishment of the symbiosis follows predictions from the physicochemical properties of the ligand-receptor interactions. Factors that alter these parameters could clearly affect symbiotic associations. Both the quantity and spectrum of Nod factors are modified by rhizosphere enzymes, thereby altering the concentration and structures of the potential ligands (Perret et al. 2000). The future use of bacterial strains and plant lines modified in the production of Nod factors or receptors, respectively, could lead to the identification of other biotic and abiotic factors influencing Nod factor signaling and the establishment of the symbiosis in the field.

Finally, it is clear that rhizobia and legumes have found a very powerful means of rhizosphere communication based on the variation in LCO structure. Oligosaccharides are much more heterogeneous then either nucleic acids or proteins and thereby provide myriad structures that rhizobia apparently exploit for host communication. How did this means of communication arise? Recent work suggests that Nod factors use a signal transduction pathway also active in establishing symbioses with arbuscular mycorrhizal fungi (reviewed in Hirsch et al. 2001), thus suggesting a mechanism in which the rhizobial symbiosis may have evolved from this more ancient and more widespread symbiosis. Nevertheless, proof of this hypothesis is lacking. Moreover, if rhizobia have exploited a pathway used by mycorrhizal fungi, have other organisms employed Nod factor signaling to establish pathogenic interactions? In this context, it is interesting to note that a nematode contains a rhizobial-like *nodL* gene (Bird and Koltai 2000), but so far no other *nod* genes essential for the production of the LCO structure have been identified in this organism. Thus, the question of whether LCO signaling is unique to rhizobia-legume communication remains unanswered.

Parasites

As mentioned earlier, phenolic molecules released by host and nonhost roots into the rhizosphere can govern germination and haustorium development in parasitic plants. Attachment of the haustorium to host roots is facilitated through mucilaginous substances at the tips of haustorial hairs that indiscriminately adhere to both biotic and synthetic surfaces (Baird and Riopel 1985). After attachment, intrusive cells at the tip of the haustorium penetrate the cortex of the host by a combination of enzymatic digestion and intrusive growth (Toth and Kuijt 1977). Because *Striga* intrusion is blocked in some *Striga*-resistant crops as well as in nonhosts, it is likely that host factors have a role at this stage, although the factors are unknown (Hood et al. 1998). The phenotypes of a few resistant crops suggest that plant–plant recognition works in both directions and that certain hosts sense the parasite's intrusion. When *Striga gesnerioides* penetrates cowpea line 58–57, a necrosis of host tissue reminiscent of a hypersensitive response surrounds the point of infection (Lane et al. 1993).

Maturation continues after the haustorim invades the host. The most obvious postcontact phenotype is the differentiation of haustorial cortical tissues into xylem strands that connect host and parasite vascular elements (Heide-Jorgensen and Kuijt 1995). The development of xylem elements is absolutely dependent upon contact with the host, implying that additional signal molecules not related to earlier haustorium development stimulate their development (Yoder 1997).

Pathogens

Pathogens also release a wide variety of chemicals that facilitate infection of their hosts (Hentschel et al. 2000). Some of these are toxins that simply kill or debilitate the plant tissue. In other cases, especially in obligate biotrophic pathogens, the developmental interaction with the host is as highly developed as in mutualists such as *Rhizobium*, although the actual signals involved have not been characterized from any biotrophic pathogens. Many pathogen signal molecules (in the broad sense) have been identified because they are recognized by plant defense receptors encoded by major resistance (R) genes resulting in host resistance and failure of infection. These pathogen molecules were identified historically from the segregation of so-called avirulence genes that are responsible for their production (White et al. 2000). However, many avirulence gene products of bacterial pathogens are injected directly into the host cell via a type-III secretion system. Thus, the role of these molecules from the pathogen's perspective is to facilitate infection, and this has been demonstrated directly in several cases (White et al. 2000). No eukaryotic counterparts to the bacterial type-III secretion system have been identified. Therefore, it is unknown whether avirulence gene products from eukaryotic pathogens including fungi, nematodes, and oomycetes such as *P. sojae* also enter host plant cells.

Nematodes

At the root surface, the site of penetration by sedentary endoparasitic nematodes is not random. For *Meloidogyne* spp. the preferred invasion sites are behind the root tip in the zone of elongation and at the site of lateral root rupture (Krusberg and Nielsen 1958). An enhanced-video-microscopy analysis showed that, on cultured *Arabidopsis*, the parasite first uses its feeding stylet to rupture epidermal cells to gain access to the host, and then migrates apoplastically towards the root tip, turning 180° before continuing into the developing vascular cylinder where stereotypical giant cells are induced (Wyss et al. 1992). These cells function as the sole and obligate nutritive source for the developing nematode. A possible role for flavonoids as signaling molecules for this behavior has been investigated. A staining approach showed elevated flavonoid levels close to the vascular system at the feeding site (Hutangura et al. 1999). However, *tt4* (2YY6) *Arabidopsis* plants, which produce no flavonoids (Burbulis et al. 1996), have normal nematode infection rates and nematode feeding sites develop at equivalent levels to those on wild-type plants (H. Koltai and D. Bird, *personal observations*). Thus, flavonoids may not be essential for the parasitic interaction.

Feeding-site induction has been discussed extensively (Bird and Koltai 2000), and although there is little doubt that an inductive signal from the parasite is involved, the nature of such a signal is completely unknown. Expression studies have shown that certain plant genes are expressed in both giant cells/feeding sites and developing rhizobial nodules (Koltai et al. 2001). Taken together, these results suggest that nodule and nematode-feeding site formation exploit shared host pathways. However, preliminary studies show *Medicago truncatula* and *Melilotus alba* mutants incapable of forming mycorrhizae or root nodules were completely susceptible to root-knot nematode infection (D. Bird, *personal observations*). These data suggest that the earliest stages of nodule development, recognition, and invasion, are not shared with those of giant cell formation.

Final Remarks and Future Directions

Plant roots bring together a diverse group of eukaryotes and prokaryotes, and in so doing transform bedrock-derived minerals into an organic-rich, living soil. Organisms that interact with plant roots communicate with multiple species using signal molecules (Fig. 1). Restricted discussions here present a minute portion of the complex, multidimensional, communication network that must exist in the rhizosphere. Much of the research on rhizosphere food webs in the past has focused almost exclusively on nutrients, particularly C, N, and P. However, diverse signal molecules function in the soil (Figs. 2 and 3), and many of these overlap to signal multiple organisms, many of which can influence mineral nutrition and growth of the plant. How this cross talk is perceived and how the signaling network is regulated are studies that need to be pursued.

We view the soil and particularly the rhizosphere as a scientific frontier where ecologists and molecular biologists can interact just as productively as the organisms that inhabit this underground environment. DNA sequence-based analysis has enabled scientists to clarify the biological complexity of soil and to uncover many new organisms, especially prokaryotes. One gram of soil contains billions of microbes, all of which are sending and receiving various signal molecules to and from the neighboring eukaryotes. The use of bacterial reporter gene-fusions as molecular sensors to track the release of nutrients and signal molecules in the rhizosphere is an important tool for future work in field ecology (Farrar et al. 2003). Utilizing certain types of transgenic plants as indicators of specific organisms or conditions in the soil will be another powerful approach for understanding the dynamics of the rhizosphere. Sophisticated physical sensors for measuring oxygen and ion levels as well as tools such as fiber optics that allow up-close views will help us understand how these underground ecosystems are structured. By learning how ''signal webs'' are established and regulated, we will better understand the dynamics of interaction among a diversity of organisms. What we need to recall is that humans are intimately associated with the 15 cm of top soil that makes up our planet's

surface. Contamination and overuse of this precious resource will not only greatly impact these signal webs and concomitantly change the dynamics of the interactions of the organisms that live there, but they will also affect us and the generations that follow us.

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