

MOLECULAR CONTROL POINTS IN RHIZOSPHERE FOOD WEBS

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Abstract. Plant roots play diverse roles in the rhizosphere. They function as organs responsible for structural support, for acquisition of mineral and water resources, and for fostering of symbiotic bacteria and fungi. They also sustain a complex food web of prokaryotes and eukaryotes in, on, and near the root. In addition to these well-known functions, roots have a recently discovered role with potentially profound coevolutionary implications for the rhizosphere food web, as well as for terrestrial ecological communities in general. Roots are both a source and a receptor of molecular signals important for mutualistic bacteria and perhaps other soil organisms. Fluid-borne rhizosphere compounds flow in the opposite direction from airborne signals that emanate from plant shoots. The powerful transpiration stream around roots naturally concentrates molecular data in a cafeteria of information about the surrounding soil. These molecular signals, such as *N*-acylated homoserine lactones and phenazines produced by bacteria, can be interpreted simultaneously by multiple species as well as by the root. We propose a model of multitrophic molecular signals in the rhizosphere that implies multiple roles of roots, as hosts, regulators, and sustainers of terrestrial productivity. We suggest a framework of regulation in the rhizosphere that functions through molecular “control points.” We define control points as regulatory elements that are operated on by selection processes to confer fitness on individual organisms and thereby have effects that propagate through other trophic levels. Identification of a control point creates a hypothesis that can be tested to assess the quantitative significance of that regulatory element. Some control points may transmit or perceive signals between organisms, but others probably integrate changing environmental conditions or external resources into individual life histories and community functions. The promise of understanding the new molecular signals is that genes must closely underlie these control points. This could offer ecology access to the power of molecular biology and allow a deep understanding of the evolutionary significance of these phenomena. One major strength of rhizospheres for addressing these issues is that realistic ecological interactions can be examined in a restricted microcosm under environmentally controlled conditions with organisms whose genomes have been completely defined and/or partially modified.

Key words: mutualism; regulation; roots; signals.

INTRODUCTION

Rhizosphere food webs are an important frontier for ecological theory where organismic biology, environmental concerns, and genomics merge. Interactions among the organisms in these food webs are crucial for sustaining natural plants and crop communities (Wall and Moore 1999, Wardle 1999). A broad base of information exists on the effects of external resources, environmental conditions, and human activity on these interactions (Moore and de Ruiter 1991, Moore 1994). More recently, a flood of strikingly detailed information on the molecular structure of rhizosphere signal compounds that are used to communicate within and between species has become available (Pierson et al.

1998, Phillips 2000). What is lacking at this point is an understanding of how these known signal factors may affect other organisms that coexist in the same environmental space. Such knowledge is important for predicting how changes in global climates or agricultural management practices can alter existing interactions between plant roots and the soil food web. We argue here that a broad view of regulatory interactions occurring through “control points” provides a robust framework for defining how signals can have unexpected, multitrophic effects. Control points must respond to selection processes and thus reflect the presence of underlying genes. Signals, which connect control points with processes, are discrete molecules that contain environmental data useful to individual organisms. In simple terms, control points are seen here as gene-dependent mechanisms that facilitate organismic survival, promote mutualisms and, possibly, frustrate antagonisms. Developing an understanding of control points in that context offers a chance to use modern

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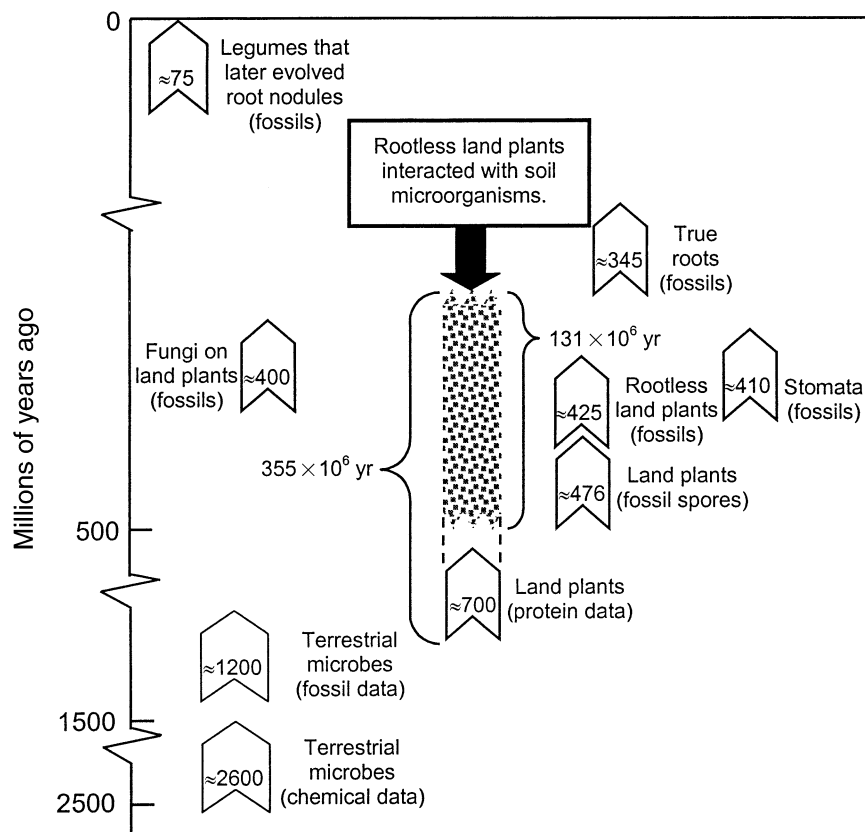


FIG. 1. Important events in rhizosphere evolution. See *Evolutionary backdrop* for details.

genomic methods to address important ecological issues.

EVOLUTIONARY BACKDROP

Plants surely encountered microorganisms in primordial soil as they moved from aquatic to terrestrial environments (Fig. 1). Geochemical evidence for microorganisms exists from 2600 million years ago (MYA; Watanabe et al. 2000), and bacterial fossils dating to 1200 MYA are known (Horodyski and Knauth 1994). Although true roots with vascular tissue appeared perhaps 345 MYA (Stewart and Rothwell 1993), early terrestrial plants had a variety of underground structures, including stems and rhizoidal appendages (Raven and Edwards 2001), which were beset early on by bacteria and from at least 400 MYA by fungi (Taylor et al. 1995). Stomata, too, were present ~410 MYA (Edwards et al. 1998), and thus water movement through the evolving soil food web toward early terrestrial plant tissues probably predated roots.

Based on this analysis, we assume that essentially all plants in early terrestrial environments interacted with microorganisms. Those relationships in primordial soil predated vascular roots by 131–355 million years, depending on whether one documents the beginning of interactions by plant microfossils (Kenrick and Crane 1997) or by estimates based on protein data

(Heckman et al. 2001). Given that the complex rhizobial symbiosis with legumes evolved in a mere 75 million years after the Caesalpinioideae group of legumes appeared (Sprent and Raven 1983), then either estimate offers sufficient time for simpler mutualisms to develop.

One cannot assess the extent to which primitive plants resisted microbial attacks, but the presence of their reasonably intact, fossilized remains shows that some protective mechanisms existed. Thus, it is reasonable to suggest that populations of epiphytic and endophytic microorganisms were an accepted fact of life for early land plants. The chemical residues of those microbial populations, as well as any signals released among the microorganisms, must have been in close contact with early land plants. Under such conditions, a sifting of water-soluble microbial products for potentially important data on the water and mineral content of nearby environments probably occurred.

RHIZOSPHERE FOOD WEBS

The complexities of rhizosphere food webs in soil today are well documented (Brussaard et al. 1997). Rhizosphere food webs contribute to mineral cycles important for plants while achieving their own biotic needs. The fact that heterotrophic soil organisms depend ultimately on plants for a source of carbon implies

TABLE 1. Traits of representative rhizosphere organisms.

Organism		Functional traits	Experimental traits	Genetic data	
Group	Example			Genome	Mutants
Plant	<i>Medicago truncatula</i>	autotrophic host	small legume	progressing	many
Root colonizers	<i>Sinorhizobium meliloti</i>	symbiont, supplies N	grows axenically in pure culture	complete	many
	<i>Pseudomonas fluorescens</i>	strong root colonizer	grows axenically in pure culture	nearly complete	many
	<i>Phytophthora sojae</i>	plant pathogen	grows axenically in pure culture	progressing	some
	endomycorrhizal fungi	symbiont, supplies P	grows on roots	limited (<i>Glomus versiforme</i>)	none
	ectomycorrhizal fungi	symbiont, supplies P	grows axenically in pure culture	none	few
Microfauna					
Protozoa	<i>Colpoda cucullus</i>	feeds on bacteria	grows on bacteria	none	none
Nematodes	<i>Caenorhabditis elegans</i>	feeds on bacteria	grows axenically in pure culture	complete	many
	<i>Aphelenchus avenae</i> <i>Meloidogyne hapla</i>	feeds on fungi feeds on roots	grows on fungi grows on roots	none some	none some
Mesofauna					
Collembola	<i>Folsomia candida</i>	feeds on fungi	grows on fungi	none	none
Enchytraeid	<i>Enchytraeus albidus</i>	feeds on fungi	grows on fungi	none	none
Mite	<i>Gamasellodes vermivorax</i>	feeds on nematodes	grows on nematodes	none	none
Macrofauna					
Insect	<i>Hepialus californicus</i>	larvae feed on roots	grows on plant material	none	none

Notes: Status of genetic information is indicated here to offer perspective on the issues addressed in this review. Preliminary genomic sequences of several other plants, including *Arabidopsis thaliana* and rice, are available, but none has the advantage of *Medicago truncatula*, which forms rhizosphere associations with a genetically defined microsymbiont.

that most of the interactions are facilitative rather than antagonistic, and many ultimately could be mutualistic (Wall and Moore 1999), either at the individual organism level or through indirect, multitrophic interactions (McCann et al. 1998). Among the best known beneficial interactions are the root-colonizing bacteria and fungi that obtain carbon substrates from plants while contributing specialized methods for acquiring important resources, such as N through N₂ fixation by Rhizobiaceae bacteria or P supplied by mycorrhizal fungi. Legumes recognize beneficial Rhizobiaceae bacteria by specific signals (Lerouge et al. 1990), but the mechanisms used by beneficial mycorrhizal fungi to penetrate protective responses of plants remain unknown. Complex interactions in biofilms, typical of bacteria adhering to abiotic surfaces (O'Toole et al. 2000), probably occur on roots and soil particles in the rhizosphere, but few data are currently available.

Trophic levels are much more difficult to resolve in rhizospheres than in aboveground ecosystems (Brussaard et al. 1997). As a result, soil organisms frequently are assigned to "functional" groups that share ecosystem roles, such as predation or N mineralization. Studies show that not all species in a functional group are required for the operation of a community (Laakso and Setälä 1999), so the exact roles of species are unclear and functional redundancies may be present. Whatever the unifying characteristics of a functional group may be, the organisms in the group share related

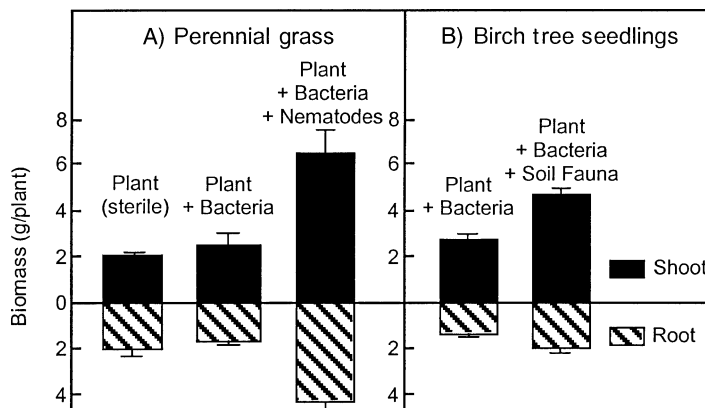
traits that depend on particular genes. One challenge, therefore, is to explain how food webs operate by relating the functional and evolutionary roles of genes in rhizosphere ecosystems (Table 1).

Carbon inputs to rhizosphere food webs are derived from living roots and soil detritus. Of these sources, root inputs have long been viewed as the foundation for the rhizosphere food web (Whipps 1990). Root-colonizing bacteria and fungi use substrates released from root hairs (Bringhurst et al. 2001) and root border cells (Hawes et al. 1998), as well as common epidermal cells. Rhizosphere microorganism currently are viewed as opportunistic users of carbon substrates emanating from roots, but new studies may identify mechanisms they possess for increasing the net efflux of organic molecules from roots.

In the past two decades, rhizosphere ecology has evolved from a subject that emphasized how organisms interacted with gross carbon flows (Lynch 1990) to a discipline that recognizes the regulatory functions of plant signal compounds in pathogenesis (Winans 1992) and symbiosis (Phillips 2000). Plant products such as flavonoids, betaines, and aldonic acids function as transcriptional regulators in root-colonizing Rhizobiaceae bacteria, but other signals from plants to rhizosphere organisms found recently (Hirsch et al. 2003) suggest a much broader role for regulatory molecules.

One factor that may distinguish rhizosphere food webs from aboveground systems is a greater prevalence

FIG. 2. Rhizosphere food webs increase plant growth. Adding nematode predators of root-colonizing microorganisms increased growth of (A) the perennial grass *Bouteloua gracilis* (Ingham et al. 1985) and (B) birch, *Betula pendula*, tree seedlings (Setälä and Huhta 1991). Increased availability of mineral N was a major factor in both responses, but unrecognized regulatory signals may have contributed to the stimulatory effects. Values are means + 1 SE.



of facilitative and mutualistic interactions (Wall and Moore 1999). Although rhizosphere food webs depend on a constant flux of organic compounds from the roots, and the plant benefits from mineral nutrients released by the food web, primitive interactions among these organisms (Fig. 1) may affect modern relations. Clearly the general “carbon out, minerals in” fluxes in the rhizosphere support the concept of potentially positive plant–food web interactions. Whether rhizospheres are characterized by an especially large proportion of facilitative plant–microbe interactions, however, may rest on the existence of a few common mechanisms that soil microorganisms use to exchange minerals for carbon. Because no such common mechanisms have been defined, the question of their prevalence remains open. Though the importance of specific mutualisms, such as mycorrhizae, is well documented, the mechanisms of their persistence are debated (Denison et al. 2003). In our minds, therefore, these facts generate a logical vision of rhizosphere food webs as collections of species interconnected by a rich array of trophic influences that range from antagonistic to mutualistic and are based on predatory impulses and signal molecules.

There is little doubt that some rhizosphere food webs promote plant growth substantially (Fig. 2). Grass plants showed a 145% increase in biomass when bacterial-feeding nematodes were added to controlled microcosms that contained plants and bacteria (Ingham et al. 1985). Similarly, soil fauna increased growth of birch seedlings by 54% (Setälä and Huhta 1991). Such results have been attributed to the increase in N mineralization associated with nematode predation on microorganisms, a concept supported by data from nematodes feeding on bacteria (Ferris et al. 1998) and fungi (Chen and Ferris 1999). This idea also is consistent with observations that increased densities of rhizosphere nematodes are associated with beneficial effects on plant growth (Wheatley et al. 1990). One question, however, is whether all of the increase in plant growth associated with the presence of nematodes can be attributed to increases in N mineralization. When similar experiments were done under varying levels of mineral

nutrition, for example, the beneficial effects of soil fauna were not completely eliminated by an increased availability of N (Laakso et al. 2000).

Not all ecologists are convinced that enhanced mineralization of nutrients is the sole explanation for increases in plant growth associated with soil food webs. Some workers have sought alternative, non-nutritional explanations (Alpehi et al. 1996), which might include either suppression of pathogens by root-colonizing organisms (e.g., Weller et al. 1988) or changes in plant growth caused by a regulatory molecule such as indoleacetic acid (Tien et al. 1979). Support for the pathogen-suppression explanation is weakened in the reports highlighted here (Fig. 2), because the beneficial effects were so large and pathogens were not reported as being present. We suggest that effects of potential regulatory factors released from food-web activities, such as passive degradation products and active signal molecules, should be considered more fully.

RHIZOSPHERE FOOD WEB REGULATION

Rhizosphere food web interactions are influenced by availability of resources such as P, other environmental factors like soil moisture and temperature, interspecific interactions, and many unknown genetic factors. Analyses of food webs have established how the presence or absence of predators and flows of energy and N can regulate fundamental rhizosphere processes (Moore and de Ruiter 1991). The presence, absence, and reproductive success of individual species have major effects on rhizosphere food webs, and thus predators are important regulators in soil food webs. These interactions have led to debates whether rhizosphere ecosystems are subject primarily to “bottom-up” or “top-down” regulation (Moore et al. 2003).

Aboveground trophic relationships also are influenced by resources, species interactions, environmental factors, and unknown genetic factors. It has long been recognized, however, that aboveground food chains differ fundamentally from rhizosphere food webs. While herbivory is the main link from a plant to the food web aboveground, the more complex belowground situation

is dominated by detritus, including root sloughings and exudates, rather than herbivory. The diverse carbon sources in the rhizosphere support multiple food channels that confound the clean dichotomy of “top down or bottom up” (Wiegert and Owen 1971). Unlike the linear food chains above ground, most consumer activity below ground is not directly connected to the plant (de Ruiter et al. 1995). Thus the top-down models of the “exploitation ecosystem” (Oksanen et al. 1981) and the HSS hypothesis (Hairston et al. 1960) are overly simplistic for the highly interconnected trophic situation of the rhizosphere (Polis and Strong 1996, Strong 1999). At the same time, it is clear rhizosphere food webs promote plant growth (Wardle 1999), and we explore here a framework for understanding how that phenomenon results from the complexity of belowground interactions.

The magnitudes of rhizodeposition and predation depend on resource availability to the plant and prey, growth rate of the plant, and growth rate of prey populations in relation to those of predators. In this sense, therefore, one might argue that either rhizodeposition or predation regulates productivity in a rhizosphere system. We seek a more molecular explanation of how rhizosphere food webs promote plant growth. To move toward that goal, we note that, just as rhizodeposition is now recognized as a process that floods the rhizosphere with regulatory signals as well as carbon substrates (Hawes et al. 1998, Phillips 2000), so too must predator-prey interactions involve the release of compounds that may have regulatory functions. These compounds, such as particular microbial products that attract nematodes, interact with other receptor sites. Both signals and receptors are products of potentially identifiable genes. We argue, therefore, that genes lie at the heart of rhizosphere food web regulation.

Genes are recognized among ecologists for their importance as both evolutionary and functional units (e.g., Mort 1991). Analyses have repeatedly linked separate genetic traits to fitness of individual organisms, species, and populations, but there has been less attention to linking genetic traits with regulatory effects on community or ecosystem functions. In rhizosphere systems, microbiologists have characterized a number of bacterial genes that influence individual life histories, populations, interspecific interactions, and even community functions (Lugtenberg et al. 2001). Effects of individual genes generally have been described or quantified by relating performance of a wild-type strain to that of a defined mutant. In some cases, the mutants were selected for an inability to produce a specific product, such as phenazine antibiotics formed by *Pseudomonas* bacteria in the rhizosphere (Thomashow and Weller 1988). In other cases, mutants were screened for impaired root colonization and then characterized to determine the nature of the genetic lesion. For example, bacterial mutants unable to synthesize thiamine (Simons et al. 1996) or certain amino acids (Simons et

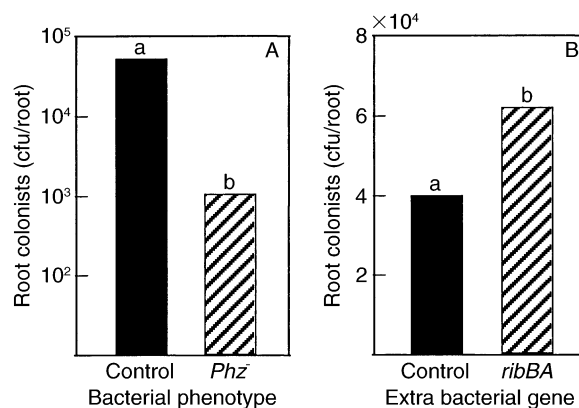


FIG. 3. Single genes affect bacterial colonization of plant roots. (A) Mutating a gene required for phenazine antibiotic production in *Pseudomonas aureofaciens* decreased wheat root colonization in soil (Mazzola et al. 1992). (B) Adding extra copies of *ribBA*, a gene contributing to riboflavin synthesis in *Sinorhizobium meliloti*, increased alfalfa root colonization (Yang et al. 2002). Significant treatment effects ($P \leq 0.05$) on colony-forming units (cfu) are indicated by different letters.

al. 1997) colonize plant roots less capably than wild-type cells. Such findings are not axiomatic because plant roots are recognized sources of vitamins and amino acids, which might support growth of bacterial auxotrophs.

Two examples show how bacterial genes affect root colonization (Fig. 3). *Pseudomonas aureofaciens* mutants unable to make phenazine antibiotics (Phz⁻ phenotype) were less capable of surviving and colonizing wheat roots than the Phz⁺ control strain (Fig. 3a; Mazzola et al. 1992). In *Sinorhizobium meliloti*, extra copies of the *ribBA* gene involved in riboflavin synthesis produced a strain that colonized alfalfa roots faster than wild-type bacteria (Fig. 3b; Yang et al. 2002). Other examples in the literature show that additional copies of nodulation genes in *S. meliloti* can enhance root nodulation and N₂ fixation on alfalfa (Castillo et al. 1999) and stimulate nodule formation by *Rhizobium tropici* on *Macroptilium atropurpureum* (Mauvingui et al. 1997). The latter case described a robust approach termed random DNA amplification (RDA), which tests random pieces of DNA to find genes that help bacteria survive under experimentally imposed conditions. Thus RDA offers a method to screen for any gene that confers a selective advantage on a rhizosphere organism through several cycles of natural selection in a soil microcosm. This technology may help locate many types of beneficial traits, possibly including genes that help soil bacteria elude nematodes or increase root exudation, because both phenotypes should be enriched through natural selection. In vivo expression technology (IVET) also offers promise for locating bacterial genes that are expressed in specific environments (Rainey 1999; Table 2).

TABLE 2. Genetic tools currently available for studying selected rhizosphere organisms.

Organism	Tool	Contribution
Plant (<i>M. truncatula</i>)	total DNA sequence (in progress)	predict gene repertoire of species; complement EST data
Bacterium (<i>S. meliloti</i>)	ESTs	detect signal effects on gene expression
	defined mutants	identify effects of known ESTs
	total DNA sequence	detect signal effects on gene expression
	defined mutants	quantify effects of known genes
Nematode (<i>C. elegans</i>)	random DNA amplification	natural selection for extra gene copies
	in vivo expression technology (IVET)	find genes expressed in particular environments
	fluorescent tags	locate defined mutants in a microcosm
	total DNA sequence	detect signal effects on gene expression
	defined mutants	quantify effects of known genes
	fluorescent tags	locate defined mutants in a microcosm

Notes: Three genetically well-characterized organisms, the legume *Medicago truncatula*, its N₂-fixing bacterial symbiont *Sinorhizobium meliloti*, and the bacterial-feeding nematode *Caenorhabditis elegans*, are suitable for testing some hypotheses discussed in this review. Although *M. truncatula* and *S. meliloti* form a symbiosis and *C. elegans* grows well on *S. meliloti*, *M. truncatula* roots have toxic effects on these nematodes (Zhao et al. 2000). Expressed sequence tags (ESTs) are nucleotide sequences, that define parts of messenger RNA transcripts in a eucaryotic organisms (Adams et al. 1991).

Bacterial genes affecting root colonization are regulated by, and can affect, more than one trophic level. Phenazine antibiotics released by *P. aureofaciens*, for example, antagonize the fungal pathogen *Gaeumannomyces graminis* var. *tritici* that attacks wheat and in the process contributes to the rhizosphere competitiveness of the bacteria (Thomashow and Weller 1988). Phenazines from *Pseudomonas* also kill *C. elegans* nematodes, which prey on bacteria (Mahajan-Miklos et al. 1999). Specific *N*-acylated-homoserine-lactone (AHL) signals from other rhizosphere bacteria regulate genes involved in phenazine synthesis (Pierson and Pierson 1996), and unidentified plant signals control AHL-regulated bacterial genes (Teplitski et al. 2000). Thus, one can ask whether phenazines benefit both the host plant and other species of rhizosphere bacteria, in addition to *Pseudomonas*, or whether pseudomonads simply evolved a regulatory system that responds to AHL molecules found commonly in many environments with bacteria, including the rhizosphere. With multiple species at different trophic levels regulating phenazine synthesis in *Pseudomonas*, one potential complexity remaining to be demonstrated is an ecologically relevant effect of phenazine on the plant itself. We suggest that such multitrophic effects of rhizosphere signals could be a little-noted characteristic of rhizosphere ecology.

MULTITROPHIC RHIZOSPHERE SIGNALS

Multitrophic chemical signals are a recognized part of aboveground ecosystems. For example, herbivorous insects feeding on plants trigger the release of volatile compounds, which influence behavior of other herbivores, carnivores, and their parasites (Dicke and van Loon 2000). Far less is known about such phenomena in the rhizosphere, but one simple comparison has profound implications. In aboveground systems, volatile factors diffuse away to infinitely dilute concentrations where the information content and, therefore, the se-

lective value become zero. In contrast, compounds released in the rhizosphere can be concentrated in soil moisture as it becomes part of the transpiration stream moving toward roots and into the plant (Fig. 4). This physical movement of water-soluble compounds to the plant root creates a natural cafeteria in which potential signal molecules can be screened by the plant for useful information content. We suggest that this differing pattern of movement in the rhizosphere favors development of multitrophic signals and may be a unique feature of this underground ecosystem.

Potential multitrophic signals in the rhizosphere could come from any interaction between food web species. External water-soluble compounds face three fates: movement toward the root, degradation, or adsorption to soil particles. If any compound elicits a response that benefits both the plant and the signal producer, then genes responsible for synthesizing the compound will be preserved in the signal-producing population. In one simple example, we posit that bacteria subjected to nematode predation produce signals that alert other bacteria to the presence of the nematodes. In their simplest form, such signals may be normal products of protoplasmic breakdown. The signals could warn bacteria in the same colony and elicit negative chemotaxis or production of a natural nematode repellent. They also could have complex effects on nematodes and mites that prey on nematodes, just as volatiles from aboveground herbivory influence arthropods and their predators (Dicke and van Loon 2000). If plants exposed to these bacterial products evolved a response that increased root elongation, the root would gain access to N mineralized by the nematode predation, while the bacteria, and indirectly the nematodes, would benefit from a larger surface area for exudation and colonization. Most biologists can suggest other testable hypotheses based on this concept. The key point is that, simply because physical forces move water toward the root, signal molecules

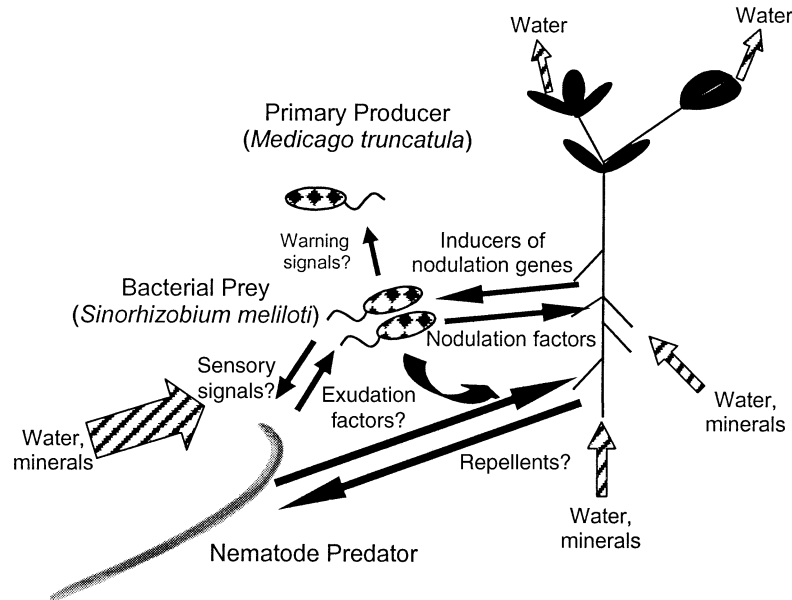


FIG. 4. Food web signal compounds may have multitrophic effects. Soil moisture in the transpiration stream moves water-soluble signals toward the root and past populations of other rhizosphere organisms. Species at other trophic levels may evolve responses that take advantage of information reflected by the presence of such signals.

produced at one rhizosphere trophic level impinge on other organisms and on the root where effects that benefit the plant and its dependent food web, will be conserved through natural selection.

Our evolutionary view of rhizosphere development (Fig. 1) argues for the presence of multitrophic signals in the rhizosphere. Organs or appendages of land plants that first explored terrestrial environments could have benefited from associations with preexisting soil microorganisms and fostered development of these interactions. Some bacteria could have prevented fungal infections; some fungi may have served as primitive root systems acquiring minerals. All living microorganisms contained moisture and a life-supporting combination of mineral elements. If AHLs or other quorum-sensing signals (Whitehead et al. 2001) were used among primitive soil microorganisms, then they, or their degradation products, are likely candidates for multitrophic signals that have effects on plants. These molecules indicated by their movement through the aqueous phase that water was available and by their microbial origin that pockets of mineral resources were present. If plants evolved a beneficial response under such conditions, then the signals would be considered multitrophic because they have regulatory effects on both bacteria and plants.

By whatever mechanisms signaling evolved between plants colonizing terrestrial environments and the preexisting soil microorganisms, roots now clearly control rhizosphere ecosystems. This dominance is based upon both the root's role as the major autotroph surrounded by a community of heterotrophs and on its evolved responses to the multitude of potential signal molecules

released by living and dead food web organisms. One challenge, therefore, is to organize these concepts into a framework that can help future research activities generate new knowledge. We suggest that rhizosphere control points offer one such context.

RHIZOSPHERE CONTROL POINTS

Defining how organisms interact, an important goal of rhizosphere research, involves understanding how resources, environmental conditions, and other organisms influence individual life histories and thereby shape characteristics of species, populations, and communities. All of these factors are important together and individually. All impinge on ecological fitness. The common thread underlying them is the mechanism by which the genome of an individual organism regulates responses to these interacting factors. For that reason, genes are a fundamental element of ecological function, just as they represent the foundation of all intracellular biology.

We offer here the concept of control points, which we define as regulatory elements operated on by selection processes to confer fitness on an individual organism. One consequence of control points is that the fitness they confer on individuals can be propagated to other trophic levels (Fig. 5). This propagation occurs, for example, because a signal released by one organism affects the function of another organism. The signal may in fact be a waste product or accidental emanation released as a result of a faulty regulatory system, but the positive benefits it offers to the producer favor its continued production. For example, classical studies with *Escherichia coli* bacteria showed that the ratio of

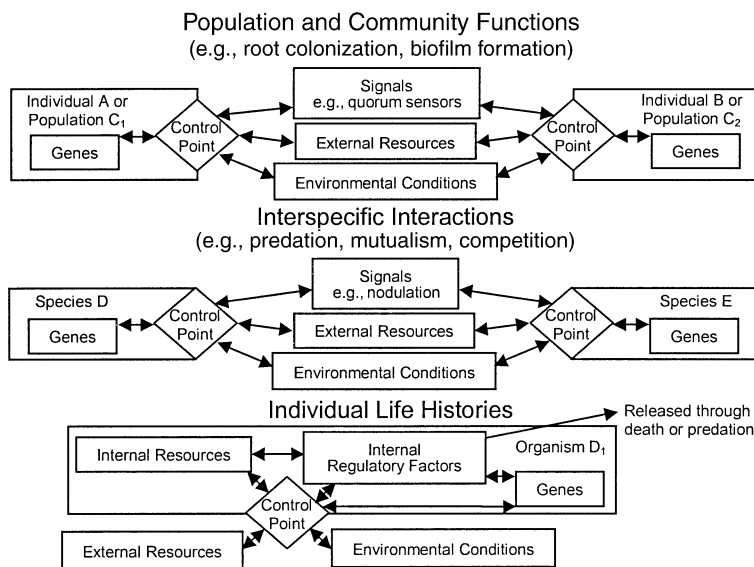


FIG. 5. Control points link signals to genes in rhizosphere ecosystems. Control points interact with external resources, environmental conditions, and external signals from living organisms. The pool of potential signal molecules in the rhizosphere is enlarged greatly by internal regulatory elements, such as vitamin cofactors, which are released through death and predation. The diversity of signals released by individuals and populations exposed to differing external resources and environmental conditions represents a molecular compendium of information for the ecosystem. Organisms that, through their control points, evolve favorable responses to valid signals from any source will have an advantage under future conditions that produce the signals.

extracellular to intracellular metabolite is only 0.01 for amino acids and 0.05 for nucleic acid bases, but the corresponding value for riboflavin varies from 0.8 to 8.0 (Wilson and Pardee 1962). If these values mirror the amounts of these molecules that are released by rhizosphere bacteria, one might suggest that flavins have the potential to be a much more important ecological factor than either amino acids or nucleic acid bases. In this example, the potential signal riboflavin together with several possible receptor sites would be viewed as multiple control points. Riboflavin has a half-life in soil of less than 72 h because of adsorption to the soil matrix and microbial degradation (Schmidt and Starkey 1951). We do not consider the soil matrix, where riboflavin adsorption occurs, as a control point because it is not acted upon by selection processes. We do, however, view genes involved in riboflavin degradation as potential control points because they are acted on by natural selection and may influence survival of individual bacteria that benefit from the ribityl group as an available carbon substrate. Possible regulatory roles of flavins in food web organisms that encounter these molecules would be other control points. Recent data show that nanomolar concentrations of lumichrome, a riboflavin degradation product from *S. meliloti* bacteria, stimulate growth of alfalfa when applied to intact seedling roots (Phillips et al. 1999). Respiration of roots exposed to lumichrome is enhanced within 24 h, which may benefit bacteria using exogenous CO₂, and a compensatory increase in net carbon assimilation can be measured as increased plant

dry mass after 12 d. Thus, these bacteria apparently have a mechanism to enhance carbon flow to the root without impairing plant growth. If other organisms, such as nematodes feeding on bacteria, have evolved a response to lumichrome, then this molecule may qualify as a multitrophic signal.

In rhizosphere ecosystems, several major regulatory control points could affect the dominant plant, while many downstream switches influence specific groups of organisms under prevailing environmental conditions. As environmental factors vary, the number of individuals in any one species in a functional group can change. Temperature, for example, which affects the abundance of different nematode species that feed on bacteria (Ferris et al. 1995), is one such environmental factor. The downstream control point in this case could be a nematode enzyme that functions more efficiently at certain temperatures. The control-point concept facilitates thinking about these functional interactions, as well as their effects on community structure, and may clarify the problem by helping to generate testable hypotheses. (e.g., a mutant lacking the control point is more/less favored than the wild-type organism.) The challenge for rhizosphere ecologists today, therefore, lies in developing a balanced clarification of these interactions that is consistent with current evolutionary, genetic, and biochemical understandings.

The seemingly broad control-point concept is narrowed by the experimentally useful fact that similar techniques can be used to compare the ecological im-

portance of many separate or linked regulatory factors. This occurs because genes are a fundamental plan for the control-point concept, while signals and their receptors interface the environment with that plan. Signals, in particular, are experimentally useful because they can be separated from the producing organism, tested on the regulated organism, and identified by modern analytical chemistry. The quantitative importance of individual signals can be assessed by mutating genes required for their synthesis or by supplying additional amounts of the signal exogenously. Signal-receptor interactions can be modified either by mutating the receptor or by supplying a chemical analogue of the signal to block the receptor. Specific, currently undefined, interactions may have major effects in rhizosphere food webs. As one example, little is known about chemical factors that alter either photosynthate flow to roots or the exudation of organic compounds (Farrar et al. 2003). All rhizosphere heterotrophs can benefit from an increase in root growth or exudation, as long as the plant remains reproductively successful. Thus there probably is positive selection for production of factors from any rhizosphere food-web species that can increase root exudation. Locating such important molecules is a central challenge for rhizosphere ecologists.

Not all control points involve a signal from another organism. Some probably comprise uptake channels for mineral resources or sensory systems that monitor environmental parameters. To understand how such control points function, the transporter protein or the environmental sensor, each a separate gene product, must be located and defined. Many tools facilitating these tasks exist among current genetic and genomic techniques (Table 2). The accessibility of these tools further justifies postulating the concept of control points because it makes the power of molecular biology available to ecologists. Defined mutants now known in plants, bacteria, and nematodes offer opportunities for quantifying the importance of specific genes under various conditions. Of potentially greater importance is the power of modern genomics. Genomics is more than isolated, or even integrated, DNA sequences. It is a blueprint defining how individual organisms, populations, and communities interact, which can be understood only when the regulation of each gene can be linked to factors emanating from beyond the transcriptional unit of the individual gene. These links constitute control points, and they can be identified by such simple experiments as identifying the presence of individual gene transcripts (ESTs, Table 2) in a root that is exposed to differing mineral resources or food web species. Identifying plant, bacterial, or nematode genes that are transcribed in response to food web changes offers ecologists a window into the formerly black box of rhizosphere ecology.

While techniques for defining control points (Table 2) can be applied to any ecosystem, rhizosphere mi-

crocosms offer optimum opportunities on several grounds. One reason is that several interacting rhizosphere species at different trophic levels already have or soon will have total genomic sequences available. A second is that, because of the minute scale of rhizosphere interactions, food webs composed of these species can be studied in realistic microcosm ecosystems under differing controlled conditions. Finally, because intact ecosystems can be examined under containment, the powerful tools of genetic modification can be applied to test ecological hypotheses safely. Taken all together, therefore, we are optimistic and excited about the future of rhizosphere biology and predict that it will generate new insights for the broader community of ecologists.

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