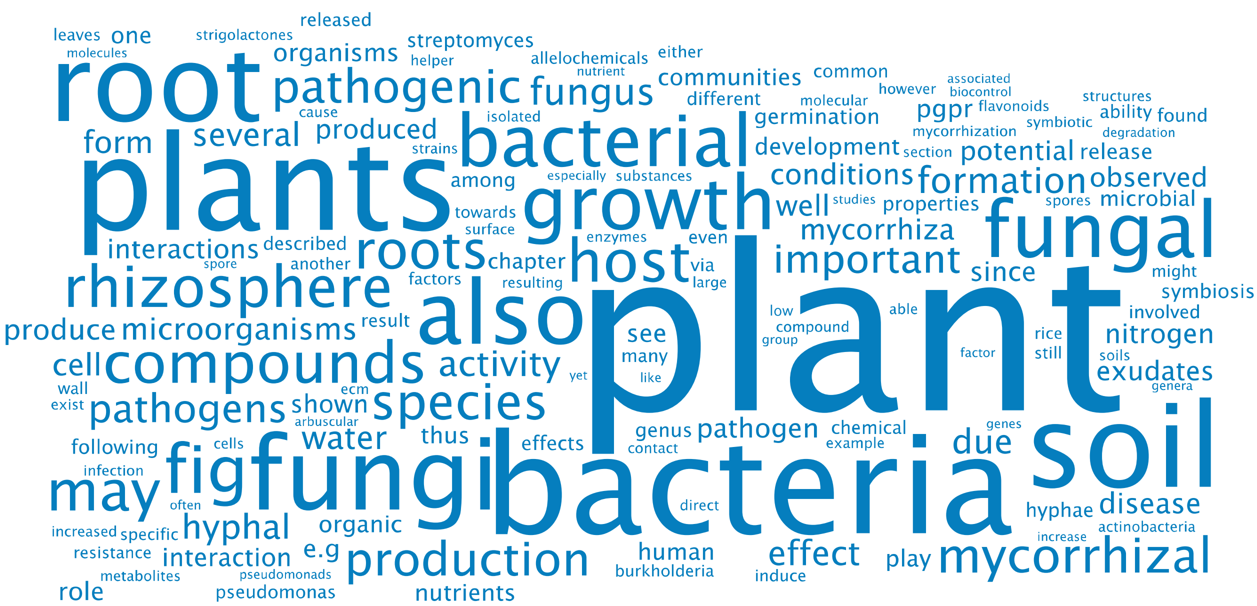

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15
Rhizosphere Interactions

Silvia D. Schrey, Anton Hartmann, and Rüdiger Hampp

Overview

Roots are important not only for water and nutrient supply of the plant, but also to release a wide range of carbon compounds of low molecular weight, such as sugars, amino, and organic acids. These can amount to between 10% and 20% of total net fixed carbon but vary based on species, nitrogen availability, and plant age. In addition, most land plants form symbioses with soil fungi, which in addition cause a considerable drain of photoassimilates. Direct (plant exudates) or indirect (via symbiotic fungi) rhizodeposition of carbon forms the basis for an environment rich in diversified microbiological populations. This was first suggested by Hiltner in 1904.

The rhizosphere is now defined as a narrow zone of soil, which is influenced by living roots. It forms a boundary layer between the root and the bulk soil. Here large fluxes of solutes and water, as well as compounds contained in the gas phase, exist. Consequently, physical soil properties can vary considerably. Depending on the demands of the plant, changes in the soil water potential can be high during the day/night cycle. In comparison to bulk soil, the soil water potential can become strongly negative during the day at high transpiration rates and less negative at night because of vertical redistribution by the root system (hydraulic lift). Special conditions also exist with regard to O_2 and pH. Following high rates of respiration by both roots and microorganisms, O_2 tension can be very low especially in wet soil where water limits diffusion rates. Uptake of solutes is often accompanied by the release of protons and organic acids, which affects the pH at the root surface.

Microorganisms of the rhizosphere establish a functional diversity that includes the decomposition of organic matter, nitrogen fixation, conversion of inorganic forms of nitrogen, solubilization of phosphate, transformation of sulfur and iron, production of siderophores (iron-binding compounds), release of plant(phyto)hormones, as well as of compounds, which are used for biotic control.

It is obvious that bacteria are an important part of the microorganisms inhabiting this ecological niche. In comparison to bulk soil, the abundance of rhizosphere bacteria is several magnitudes higher ($10^{10}-10^{12}$ microbes per gram soil versus $<10^8$ in bulk soil), but still about 100 times lower than under culture conditions. Bacteria can solubilize nutrients from the mineral soil layer, but will also sequester them. Consumption of bacteria by soil protozoa and nematodes will then liberate nutrients, which in due course will become available for plants.

Fungi form another important part of the rhizosphere. Most terrestrial plants develop symbiotic structures (mycorrhiza) with soil-borne fungi, creating another sphere, the mycorrhizosphere. In these interactions, the fungal partner provides the plant with improved access to water and soil nutrients because of more or less complex hyphal structures, which emanate from the root surface and extend far into the soil. The plant, in return, supplies carbohydrates for fungal growth and maintenance. Because of leakage and the turnover of mycorrhizal structures, these are another source for solutes released into the soil where they can be accessed by other microorganisms.

In the following interactions of bacteria, fungi and plants, and, finally those of plants with each other are addressed. With regard to soil bacteria, a wide range of bacterial activities exist such as the "good" ones (plant growth promotion, plant disease suppression, nitrogen fixation) and the "bad" ones (plant pathogens), as well as bioactive compounds of bacterial secondary metabolism, which cause the respective effects. Plant-associated bacteria act as opportunistic human pathogens. Fungi form another focus, here especially the bacterial influence on symbiotic and plant pathogenic fungi. Finally, direct (parasitic plants, plant competition) and indirect (by the help of fungi) interactions of plants themselves are described.

The examples introduced below show that the contemporary knowledge about organisms and interactions in the rhizosphere has increased recently, mainly because of the many attempts to improve plant growth and fitness.

Ecological Biochemistry: Environmental and Interspecies Interactions, First Edition. Edited by Gerd-Joachim Krauss and Dietrich H. Nies.

Companion Website: http://www.wiley.com/go/Krauss/Nies/EcologicalBiochemistry

The intense use of metagenomics with soil samples continuously reveals the enormous diversity of microorganisms living and thriving on plant-derived exudates. Interestingly, most of these organisms are not pathogenic in nature. This is understandable because they depend on the continuous delivery of organic compounds by the plant. This could also explain why so many rhizosphere bacteria produce toxins that mainly affect plant pathogenic microorganisms or release compounds that are plant-beneficial.

An investigation of the chemical interactions is, however, extremely limited owing to the lack of suitable experimental procedures and of set-ups that represent realistic conditions. Experimentally sound studies can most easily be performed under sterile conditions using cocultures of the respective organisms or exuded compounds. Three partite systems (host plant + two microorganisms (e.g., pathogen + antagonist)) become already very difficult to handle, and the step to field studies is enormous. Many other organisms now interfere, metabolically modifying the "identified bioactive compounds" to an unknown extend. Further, owing to different binding properties of soil particles and other soil chemical factors, the fate of any such compound remains unclear. Thus, there is still a long way to go to broaden our understanding of the processes and molecules involved in rhizosphere interactions.

15.1 Bacterial Communities in the Rhizosphere

Characterization of rhizosphere bacteria was for a long time possible only for culturable microorganisms. With the advance of sampling and molecular techniques, the ecology of rhizosphere microorganisms becomes accessible and several overviews on rhizosphere microbes of important crop plants based on 16S rDNA and 16S rRNA have been produced in recent years (Table 15.1). The structure of soil microbial communities varies largely in response to the plant cover, the soil type, and the history of the soil (arable land under rotation, monoculture, or permanent grassland).

Root exudates are an important factor for establishing microbial communities in the rhizosphere, however, exudation along roots is not homogeneous. Abundance and turnover of rhizobacteria are further regulated by microfaunal grazers such as protozoa. Consequently, beneficial effects of protozoa on plant growth have been related to nutrients released from consumed bacterial biomass (Figure 15.1). Higher spatial resolution shows that bacteria accumulate in certain areas, indicating a possibly higher exudation activity. The zone of root elongation is especially effective in attracting a large diversity of microbes. Fluorescent pseudomonads, common bacterial inhabitants of rhizospheres, preferably colonize the root base and to a lesser extent the younger root areas. Further attractive sites are the penetration points of lateral roots and around the growing root tip with the mucilage forming root cap, which offers easy access to nutrients.

The ability of rhizosphere bacteria to consume specific root exudates determines the bacterial colonization pattern of roots. By altering root exudate composition or by the production of specific exudates, plants can steer the microbial rhizosphere population. This might explain the similarity of the community structures of members of one plant species growing in different soils and of different plants in the same soil harboring very different bacterial communities. When under attack, plants may alter their root exudates composition in a way that attracts beneficial microbes. Thus, the two factors, soil type and plant species, have a major effect on rhizosphere-associated microbial communities.

Comparable to bacterial colonization of root surfaces, leaves, which are the aboveground plant surfaces termed **phyllosphere**, are commonly colonized by a diverse community of bacteria (Figure 15.2). Bacterial aggregates form at the depressions between adjunct epidermal cells, along veins and at the bases of trichomes (hairs). Usually, these bacterial aggregates form biofilms and are embedded in an EPS matrix (extracellular polymeric substances, see Section 14.5.1). The EPS might be helpful to maintain a hydrated surface because life in the phyllosphere requires adaptation to abiotic stresses. Few bacterial phyla predominate in the phyllosphere of different plants; plant factors are involved in shaping these phyllosphere communities. *Proteobacteria*, specifically *Alpha-* and *Gammaproteobacteria,* predominate the phyllosphere of distinct plant species. However, in contrast to observations of active plant recruitment of specific bacterial communities for rhizosphere colonization, such an effect has yet to be shown for the phyllosphere.

15.1.1 Plant Growth Promoting Rhizobacteria

Soil samples can contain a high proportion of plant growth promoting microorganisms, which may amount up to two-thirds of those that can be cultivated. Plant growth promotion by microbes can be direct by improved nutrient availability ("**biofertilizers**") and hormonal stimulation and increased resistance against pathogens but also indirect by controlling the growth of plant pathogenic organisms ("**biopesticides**" (Figure 15.3)).

Plant growth promoting rhizobacteria (PGPR) are usually in contact with the root surface as well as the

Table 15.1 Microbial community in the rhizosphere of agriculturally important plant species.

Plant species	Rhizosphere-dominant species
Beta vulgaris (*Caryophyllales*, core eudicotyledons)	*Proteobacteria*, *Bacteroidetes*/*Chlorobi* group
Brassica napus cv. Licosmos (*Brassicales*, *Rosidae*)	*Actinobacteria*, *Proteobacteria* (α, γ), *Firmicutes*
Brassica napus cv. Westar (*Brassicales*, *Rosidae*)	*Proteobacteria* (α, β, γ), *Bacteroidetes*/*Chlorobi* group
Dendranthema grandiflora cv. Majoor Bosshardt (*Asterales*, *Asteridae*)	*Firmicutes* (*Bacillus*), *Proteobacteria*: α (*Acetobacter*, *Azosporillum*), β (*Comamonas*, *Ralstonia*, Variovarox), γ (*Pseudomonas*),
Fragaria ananassa (*Rosales*, *Rosidae*)	α-*Proteobacteria*, *Actinobacteria*
Hordeum vulgare cv. Pastoral (*Poales*, *Liliopsida*)	*Firmicutes* (*Bacillus*), *Proteobacteria*: β (*Burkholderia*), γ (*Acinetobacter*, *Pantoea agglomerans*, *Pseudomonas*),
Lolium perenne cv. Bastion (*Poales*, *Liliopsida*)	*Firmicutes*, *Proteobacteria*: α, γ (*Pseudomonas*)
Medicago sativa (*Fabales*, *Rosidae*)	*Proteobacteria*: α, γ, *Firmicutes Bacteroidetes*/*Chlorobi* group
Persea americana (*Laurales*, *Magnoliidae*)	*Proteobacteria* γ (*Pseudomonas*), δ (*Polyangium*)
Phaseolus vulgaris (*Fabales*, *Rosidae*)	γ-*Proteobacteria*, *Bacteroidetes*/*Chlorobi* group
Pinus contorta (*Coniferales*, *Coniferophyta*)	*Proteobacteria*: α, β, γ, *Acidobacteria* (*Acidobacterium*)
Solanum tuberosum (*Solanales*, *Asteridae*)	*Proteobacteria*: α, γ, *Firmicutes* (*Bacillus megaterium*)
Trifolium pratense (*Fabales*, *Rosidae*)	*Proteobacteria*: α, γ
Zea mays (*Poales*, *Liliopsida*)	*Proteobacteria*: α (*Rhizobia*), β (*Burkholderia*), γ, *Bacteroidetes*/*Chlorobi* group

From Hawkes, DeAngelis, and Firestone (2007), modified and taxonomic information added.

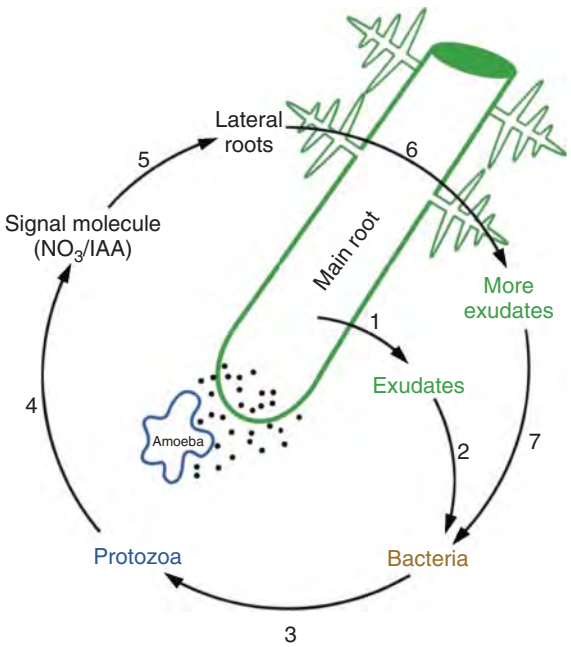


Figure 15.1 Microbial loop in soil: bacteria accumulate at sites of prolific root exudation (1,2). Protozoa feed on those bacteria (3), in return releasing nutrients and signal molecules (4). Lateral root formation is increased resulting from signaling molecules (5), resulting in even more root exudates (6) and thus an increase in the amount of bacteria. (Modified from Bonkowski (2004).)

hyphal cell walls of symbiotic fungi. PGPR may support plant growth by the mobilization of inorganic nutrients, by nitrogen fixation, or by the production or degradation of phytohormones including auxins, cytokinins, or gibberellines. Volatile substances produced by bacteria, such as acetoine or 2,3-butanediol (see Section S1.3.3.5), can also stimulate plant growth substantially (Figure 15.4). Some root-associated bacteria, among them pseudomonads and enterobacteria (*Gammaproteobacteria*), are able to stimulate plant growth by reducing inhibitory levels of ethylene in the rhizosphere through the hydrolysis of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid. These root-associated or endophytic bacteria have a good potential for practical applications. In soils with low phosphate availability, bacteria release phosphate from minerals and organic phosphate sources. Although many P-solubilizing bacteria have been characterized, their relative importance in the PGPR effect is uncertain. However, if the phosphate ions are released in an area rich in mycorrhizal fungal hyphae, the hyphae may transport phosphate to the plants and the PGPR effect is detectable, emphasizing the importance of interaction in the rhizosphere between more than two partners.

Azospirillum strains (*Alphaprotobacteria*) are known mainly for their ability to fix atmospheric nitrogen. The

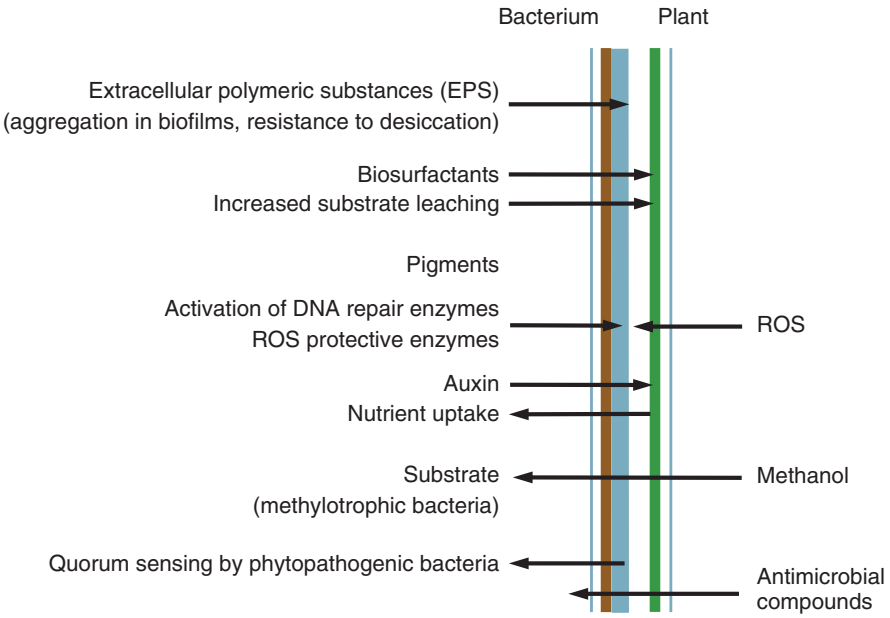


Figure 15.2 Phyllosphere: Plant–bacteria interactions. (Graphics: D. Dobritzsch, G.-J. Krauss.)

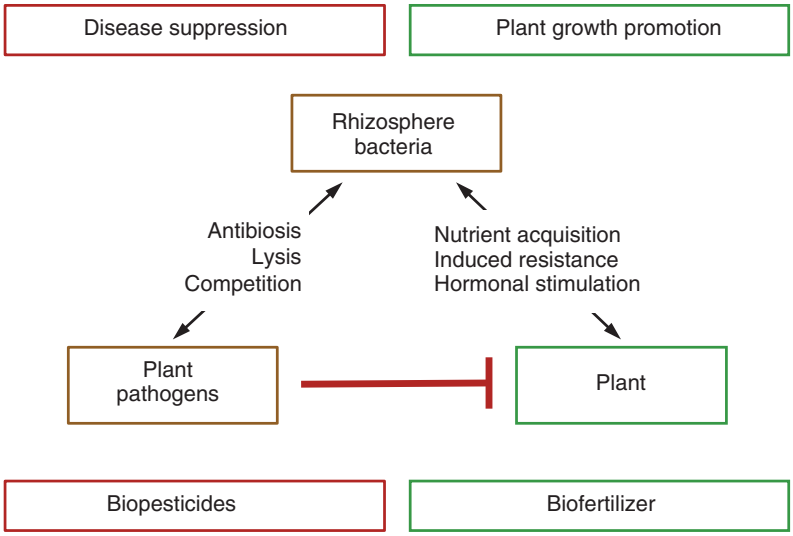


Figure 15.3 Plant growth promoting rhizobacteria can be separated into biopesticides that suppress diseases by antagonism of pathogenic microorganisms and biofertilizers that promote plant growth by exerting a direct effect toward the plant. (Modified from Berg (2009).)

2,3-butanediol Acetoine (3-hydroxybutanone)

Figure 15.4 Plant growth stimulating volatiles.

inoculation of roots with *Azospirillum*, however, often promotes plant growth not primarily through nitrogen fixation, but also because of the ability of the bacteria to produce phytohormones that stimulate root development, increase the volume of explored soil space and eventually improve nutrient uptake efficiency. The auxin-type phytohormones produced by *Azospirillum* induce root branching and thus improve plant nutrient uptake from the soil, an example for a possible trade-off of altered resource allocation. However, it has to be kept in mind that PGPR functioning can rarely be reduced to a singular bacterial trait but to a combination of many mechanisms, either by one or several microorganisms acting in concert.

Inoculation of plants with some PGPR elicits a phenomenon known as **induced systemic resistance** (ISR), Figure 15.5). ISR allows the plants to endure pathogen attacks that, without bacterial preinoculation, could be

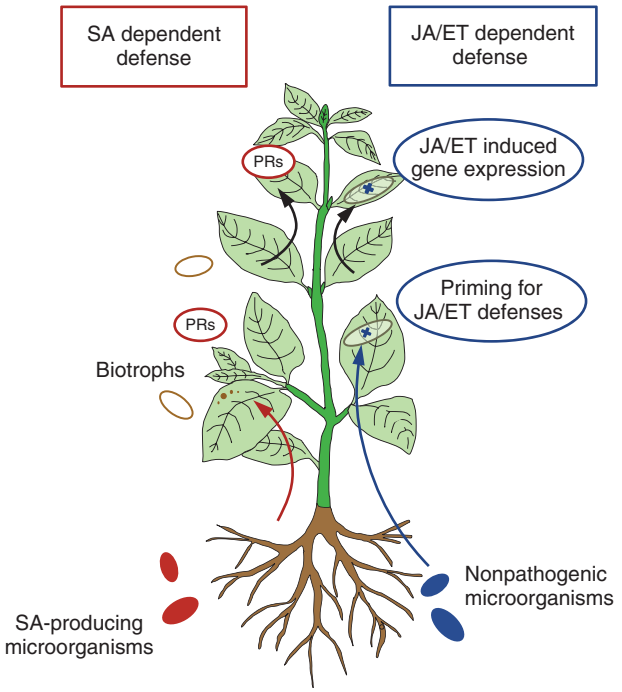


Figure 15.5 Illustration of the induction of systemic resistance in plants. Left: salicylic acid-dependent defense responses develop following infection with certain biotrophic pathogens or SA-producing soil microbes in uninfected (distal) tissues and are characterized by expression of pathogenesis-related (PR) proteins. Right: Jasmonic acid/ethylene (JA/ET)-dependent defenses can be initiated by root-associated soil bacteria and become evident following aboveground pathogen infection by the expression of JA/ET-dependent genes. JA/ET-dependent gene expression is also relevant following infection with certain necrotrophic pathogens. Both types of induced resistance are effective against a broad range of plant pathogens. (Adapted from Pieterse *et al.* (2009).)

lethal. The effect is systemic, for example, root inoculation with the biocontrol PGPR yields distal plant parts like leaves non- or less susceptible to attack by the respective pathogen. Many bacterial (and fungal) genera have thus far been shown to elicit ISR, among them *Pseudomonas*, *Burkholderia*, *Bacillus*, and *Streptomyces*. Several molecules have been determined to induce ISR. For example, root treatment of *Phaseolus vulgaris* with a *Pseudomonas putida* strain lead to a significant reduction of the disease caused by the pathogenic fungus *Botrytis cinerea* on leaves. The isolated molecular determinant of *P. putida* mainly responsible for ISR was identified as a polyalkylated benzylamine structure (Figure 15.6). Exposure to the volatile compound 2,3-butanediol that promotes growth of *Arabidopsis* seedlings also results in a decreased disease severity following infection with the bacterial pathogen *Erwinia carotovora*. Transgenic lines of *Bacillus subtilis* that emitted reduced levels of **2,3-butanediol** (Figure 15.4), decreased *Arabidopsis thaliana* protection against pathogen infection compared with seedlings exposed to volatiles from wild-type bacterial lines. Furthermore, bacterial signaling molecules of the ***N*-acyl homoserine lactone** type (Figure 15.7), which are quorum-sensing compounds of Gram-negative bacteria, were found to exert systemic functions in plants as well.

15.1.2 Plant Disease Suppression by Rhizobacteria – Indirect Plant Growth Promotion

Infectious diseases are often caused by soil-borne organisms including both bacteria and fungi. These pathogens can infect plants directly via the roots or by emanating spores or other infectious particles that may also infect aboveground plant parts. Yet, under natural conditions **disease incidence**s are rare. This has been linked to the fact that between 1% and 35% of microbial isolates from plant-associated soil samples possess antagonistic activity against plant pathogens when tested in *in vitro* cultures.

Soils, where soil-borne diseases are infrequent despite the presence of the pathogen and susceptible host plants, are called *suppressive soils*. They show the capacity to decrease disease incidence. This phenomenon can be observed following disease severity monitoring after inoculation of sterilized soil with a pathogen compared to disease development in nonsterilized soils. Mechanisms behind this effect have been related to the activities of the soil microbial population during a time critical for the development of the pathogen. Here, effects of individual groups of microorganisms against a specific pathogen may play an important role because disease suppression is often caused by specific bacterial and fungal populations. Such naturally occurring antagonistic organisms express traits that enable them to interfere with pathogen growth, survival, and infection. Bacteria that are antagonistic to plant pathogens, represent an important part of the rhizosphere communities, and antagonistic strains amount up to 35% of the cultivable bacteria.

The most thoroughly investigated group of PGPR antanists are still the fluorescent pseudomonads. These bacteria produce several metabolites that suppress the growth of other organisms. For example, the extracellular pigment **pyoverdin** (Figure 15.6) is an efficient **siderophore** (iron carrier), and the production of pyoverdin by pseudomonads in iron-poor soils is an effective way of suppressing the growth of nonproducers by depriving the pathogens from iron. Pseudomonads also produce metal chelating agents with proposed properties other than iron scavenging. The siderophore **pyochelin** (Figure 15.6), for example, effectively binds copper and zinc, and possesses strong antimicrobial activity. The antimicrobial effect of pyochelin, and of some other siderophores, can be explained by their effective metal chelating activity. Gram-positive PGPR antagonists, like several *Bacillus* and *Streptomyces* (*Actinobacteria*) species, are also very efficient PGPR-strains with biocontrol activity, and also have effects on systemic resistance in plants. Because their

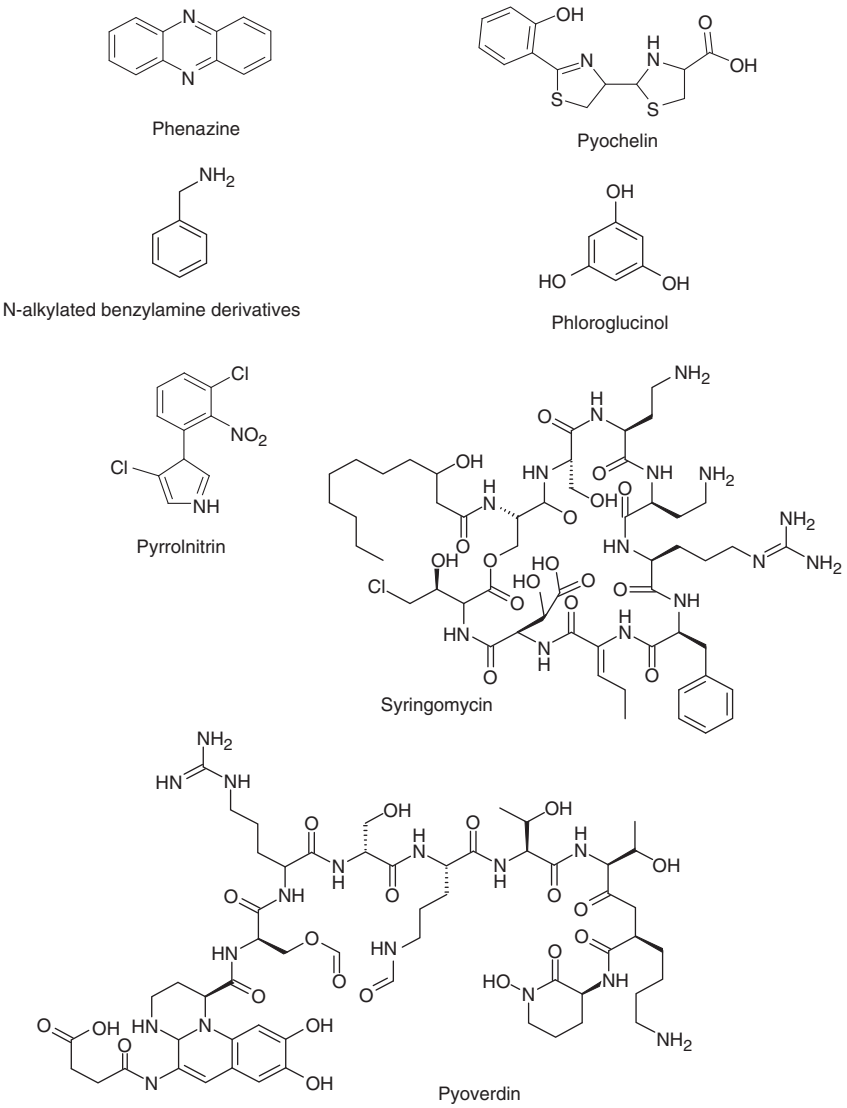


Figure 15.6 Compounds involved in plant disease suppression.

spores withstand adverse conditions, they have wide acceptance for practical application, especially following easier handling and excellent stability of inoculant preparations.

Direct **antibiosis** is used by several PGPR as a mechanism for **biocontrol**. Antibiosis by PGPR pseudomonads is often caused by the production of antimicrobial substances. These chemicals do not only suppress fungi, but are also often toxic against other bacteria. From antimicrobial compounds produced by pseudomonads, the mode of action has been partly determined for several classes of substances. These include the electron transport inhibitors **phenazines**, **phloroglucinols** (causing membrane damage in *Pythium* spp. (*Oomycetes*, *Stramenopiles*) and being phytotoxic at higher concentrations), **pyrrolnitrin** (acting as a fungicide), cyclic lipopeptides (surfactant properties against fungi and plants, chelation of cations), and **hydrogen cyanide** HCN (potent inhibitor of metalloenzymes), (Figure 15.6). Production of siderophores, lipopeptides, and antibiotics production has been observed in other PGPR isolates as well, including *Bacillus amyloliquefaciens*, *Stenotrophomonas* spp. (*Gammaproteobacteria*), and *Streptomyces* spp.

Another group of antagonistic compounds are lytic enzymes, such as cell wall hydrolases that attack pathogens. The ability to degrade fungal cell walls by chitinases is shared by many biocontrol PGPR including *Pseudomonas*, *Serratia,* and *Streptomyces* spp. In addition to chitinases, some bacterial strains produce ß-glucanases and proteases. Synergism between the action of cell wall degrading enzymes and antibiotics was observed. For example, it was shown that the pretreatment of plant pathogenic fungi with cell wall degrading enzymes rendered them more susceptible to the antifungal substance **syringomycin** (Figure 15.6).

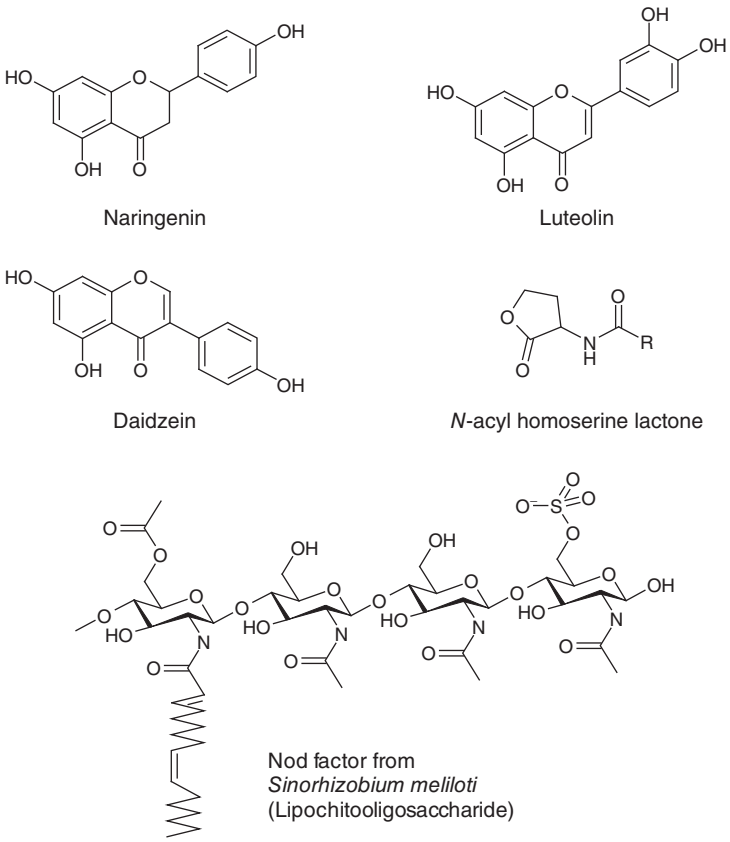


Figure 15.7 Root nodule formation related compounds. The flavonoids luteolin, naringenin, and daidzein induce *nod* gene expression in bacteria, the bacterial nod factors modulate root reorganization. *N*-acyl homoserine lactone functions in bacterial density measurement (quorum sensing).

15.1.3 Nitrogen-Fixing Plant–Bacterium Symbiosis

Certain bacteria form symbiotic structures with plants. The best-studied example is nitrogen assimilation in leguminous plants via an endophytic symbiosis with saprophytic free-living bacteria in the soil belonging to **Rhizobiales** (Genus *Rhizobium*; *Alphaproteobacteria*). They show a considerable phylogenetic diversity, which is also reflected by distinct metabolic properties. On roots of host plants, rhizobia induce the formation of organs called *nodules*, which are then colonized intracellularly. Here, they reduce nitrogen to ammonia, which can subsequently be used by the plant (see Section 5.2.2).

The establishment of this plant–bacteria interaction is regulated by chemicals. Relevant bacteria have to reach a certain population density before they are able to infect the root. Determination of this density is done following quorum-sensing (***N*-acetyl homoserine lactones**, AHLs, Figure 15.7). AHLs regulate gene expression programs in the bacteria, which are a prerequisite for symbiosis establishment. The mutual recognition of both partners of the symbiosis starts by the release of flavonoids (e.g., **naringenin**, **daidzein**, **luteolin**) by the host plant that direct bacteria toward the host root. These flavonoid signals not only attract relevant bacteria but also induce bacterial gene expression resulting in the production of so-called **nod-factors** (**lipochitooligosaccharids**) by the bacteria (Figure 15.7) (see Section 5.2.2.1).

Growth of rhizobia and nodule formation can be influenced by other soil bacteria, like streptomycetes (*Actinobacteria*). Antibiotic production by these may cause unsuccessful nodulation or even inhibit nodulation under field conditions.

Actinobacteria may not only influence root nodule formation with bacteria belonging to the genus *Rhizobia*, but may also fix nitrogen themselves. Within this bacterial phylum, members of the genus ***Frankia*** are able to fix atmospheric nitrogen either in a symbiosis with several dicotyledonous plants (e.g., *Alnus* species, *Fagales*, *Rosidae*), collectively called **actinorhizal plants** but, in contrast to rhizobia, also in nonsymbiotic state under aerobic conditions. The similarities between signaling in arbuscular mycorrhiza (AM) and legume/rhizobia symbiosis and the common ancestry of rhizobia and *Frankia* host plants indicate also a similarity in the use of signaling compounds. Even though the first sequenced genomes of *Frankia* did not reveal gene clusters homologous to rhizobial common

nod genes, it can be expected that *Frankia* Nod factor equivalents share similar features, that is, chitin-based signals (lipochitooligosaccharides) that are perceived by plant receptor kinases.

Cyanobacteria are a prokaryotic phylum with many species able to fix nitrogen. Some cyanobacteria form associations with a wide range of host plants belonging to the ferns (*Polypodiosida*), liverworts (*Marchantiophyta*), hornworts (*Anthocerophyta*), or plant families *Cycadaceae* (*Cycadales*, *Cycadophyta*) and *Gunneraceae* (*Gunnerales*, core eudicotyledons). The cyanobacterial symbionts found in these associations mainly belong to the genus **Nostoc** (*Nostocales*). These bacteria are characterized by possessing a few nitrogen-fixing cells (called **heterocysts**), resting spores called **akinetes**, and filaments that play an important role in the infection process. These so-called hormogonia are short gliding filaments that migrate rapidly into the preformed cavities of the future host plants. To trigger this event, host plants produce **hormogonia-inducing factors (HIF)**, yet the nature of these signals is still unknown. Once symbiosis is established, hormogonia production is again suppressed by as yet unidentified plant-produced hormogonia-repressing factor.

A special form of cyanobacteria symbiosis was observed in the association with the fungus *Geosiphon pyriformis*. *Geosiphon pyriformis* belongs to the *Glomeromycota* (*Fungi*, *Opisthokonta*), the same fungi that form AM and have thus attracted interest from the field of AM research (see Section 15.2.1 and Chapter 5). For *Geosiphon pyriforme* this symbiosis is obligate while the partner *Nostoc punctiforme* can be cultivated without the fungus. To establish the symbiosis, *Nostoc* and *Geosiphon* get into contact on the soil surface where the fungal hyphae surround the filaments of *N. punctiforme* and eventually incorporate them. The resulting structure is a bladder of up to 2 mm length in which the bacteria reside. Until now this is the only known fungus–cyanobacterium endosymbiosis.

15.1.4 Actinobacteria: Prolific Producers of Natural Compounds

Actinobacteria are among the most numerous bacteria in rhizospheres and play important roles in the decomposition and turnover of even the most recalcitrant organic materials such as cellulose, chitin, and lignin. Within the bacterial phylum *Actinobacteria* the order *Actinomycetales* comprises several genera that are characterized by the ability to produce a great variety of secondary metabolites. Members of the genus *Streptomyces* in particular, have developed an efficient machinery for the production and modification of secondary metabolites with antagonistic properties, probably because of their manifold interactions with other rhizosphere microorganisms. Also, the ability of streptomycetes to act as PGPR has recently attracted increased attention. Streptomycetes are generally saprophytic organisms that spend the majority of their life cycles as semidormant spores, especially under nutrient-limited conditions. When spores germinate, they produce a substrate mycelium, which uses extracellular hydrolytic enzymes to degrade organic compounds that usually resist degradation by other microbial groups, such as plant and fungal cell wall polysaccharides, and insect exoskeletons. The substrate mycelium fragments into chains of spores during maturation.

Streptomyces strains can be distinguished by their ability to produce secondary metabolites effective in organismic interactions (Figure 15.8). Several reports have shown how the biological activity of such secondary metabolites relates to their biocontrol activity. Streptomycetes produce bioactive compounds with antifungal and antibacterial activity, for example, 2-methylheptyl isonicotinate, which suppresses dominant soil-borne phytopathogenic fungi belonging to the genera *Fusarium* and *Rhizoctonia*. Such substances might exert their effect even when inoculated on seeds of cruciferous plants, resulting in resistance to fusarial wilt of crucifers. If both culture filtrate and spore suspension of such streptomycetes exhibit protective activity, they may be promising biocontrol microbes. Other compounds, like aminoglycoside antibiotic paromomycin, which inhibits the *in vitro* growth of severe oomycete plant pathogens (*Stramenopiles*) from the genera *Phytophthora* and *Pythium*, and which shows potential *in vivo* activity against red pepper and tomato late blight, have been described.

15.1.5 Plant Pathogenic Soil Bacteria

Compared to fungal plant pathogens there are relatively few soil-borne plant pathogenic members among bacteria, which belong to different subphyla of the *Proteobacteria*. Among those are the well-studied *Ralstonia solanacearum* (global distribution with unusually wide host range, causes wilt diseases, *Betaproteobacteria*), *Agrobacterium tumefaciens* (crown gall disease, *Alphaproteobacteria*), or the gammaproteobacterial genera *Xanthomonas*, *Pseudomonas*, and *Erwinia*. (*Erwinia amylovora* causes fire blight on apples, *Erwinia chrysanthemi*, *E. carotovora* soft rot). Xanthomonads cause disease symptoms in important crop plants, for example, raised circular lesions on leaves, which later on turn into brown corky cancer. They use a type III secretion system to secrete virulence effector proteins into the plant cell cytosol, which then modulate plant gene expression. *Pseudomonas* strains can be plant beneficial, but others, like *Pseudomonas syringae,* can also infect many taxonomically diverse plant species. The pathogenic ones also make use of the type III secretion system to inject proteins into the host cells. In addition, they produce the polyketide toxin, **coronatine** (Figure 15.9), which is required for full virulence and is similar to the phytohormone methyl jasmonate.

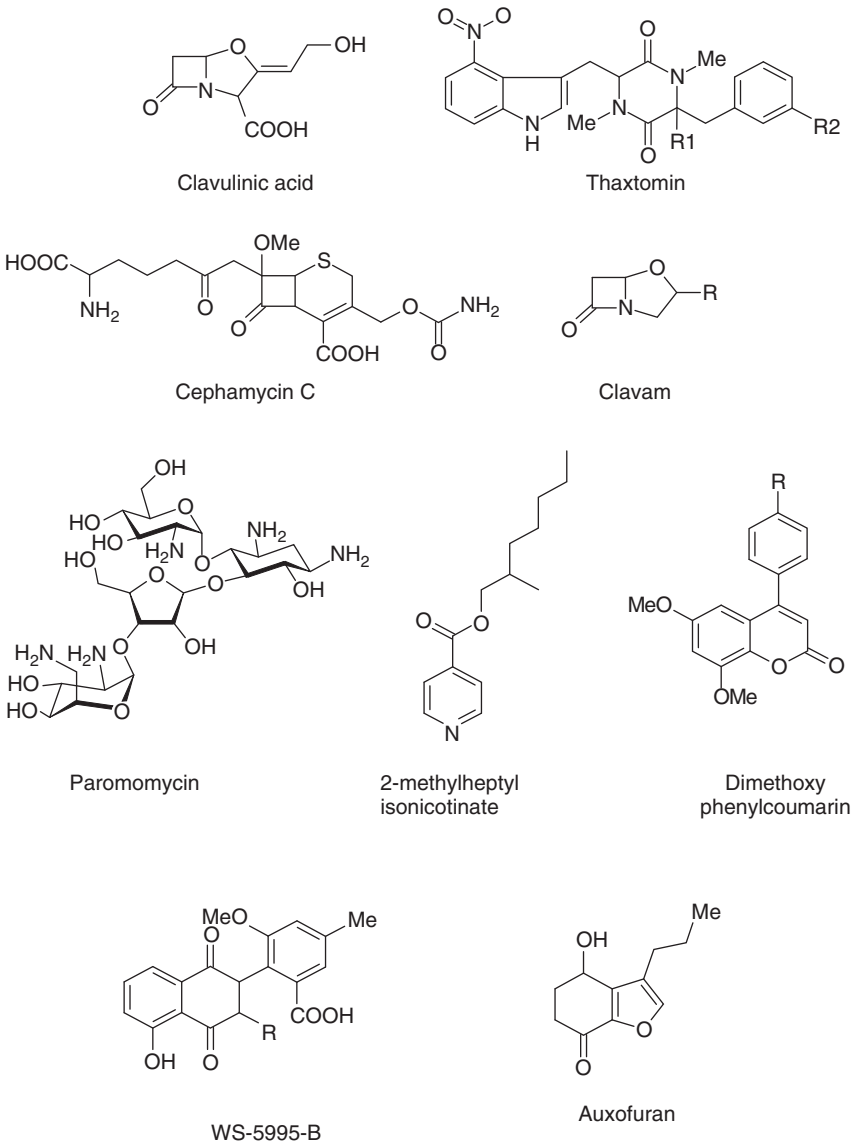


Figure 15.8 Secondary metabolites from streptomycetes.

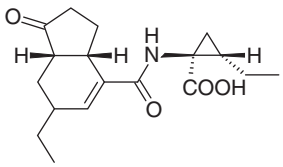


Figure 15.9 Coronatin, a polyketide produced by the plant pathogenic bacterium *Pseudomonas syringae*.

Among the hundreds of the described *Streptomyces* species, to date, only a few have been identified as plant pathogens. These species, *Streptomyces scabies*, *Streptomyces acidiscabies*, *Streptomyces turgidiscabies*, and *Streptomyces ipomoeae*, cause the common scab disease in potato and other taproot crops (reduction of root and shoot length, intense radial swelling of roots, tissue chlorosis, and necrosis). The mechanism of pathogenicity has been related to the production of a family of cyclic dipeptides, **thaxtomins**, by the streptomycete (Figure 15.8). Plant pathogenic *Streptomyces* species possess a conserved biosynthetic pathway for thaxtomin, which is essential for disease development.

15.1.6 Plant-Associated Bacteria as (Opportunistic) Human Pathogens

Recent mass infections such as **EHEC (enterohemorrhagic *E. coli*)** show the potential of plant-associated bacteria to have human hosts, or *vice versa*, that human pathogens thrive on plant surfaces. Among the rhizosphere bacteria with the potential to be opportunistic human pathogens, species of the genus *Staphylococcus* (*Firmicutes*) or the proteobacterial genera *Burkholderia* (β), *Enterobacter* (γ),

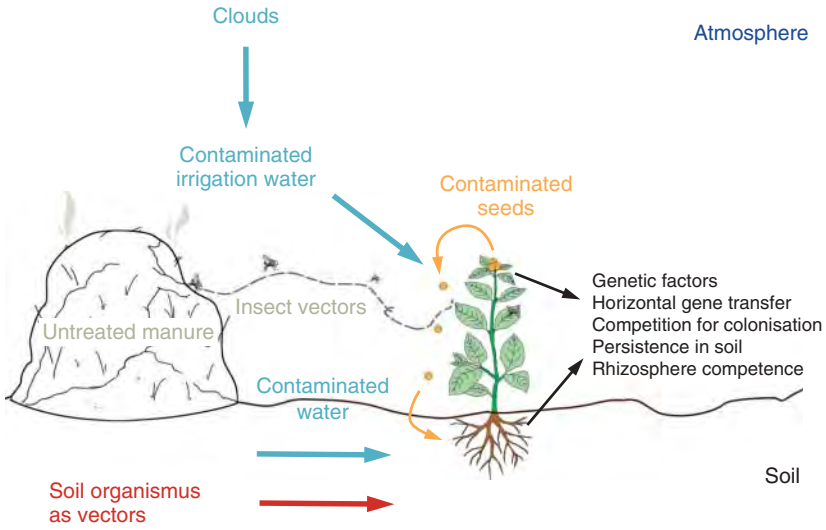


Figure 15.10 Mechanisms of spread and persistence of human pathogenic microorganism in the rhizosphere (Reproduced from Brandl (2006) with permission of Annual Review of Phytopathology.)

Herbaspirillum (β), *Ochrobactrum* (α), *Pseudomonas* (γ), *Serratia* (γ), and *Stenotrophomonas* (γ) can be found.

Animal produce was typically regarded as a main source of infection with **human pathogenic bacteria** while pathogens on fruit and vegetables are considered mostly a postharvest contamination rather than a contamination in the field. Increased reports of outbreaks associated with fresh produce have raised questions about the fitness of pathogens on plant surfaces, not only aboveground, but also belowground. Contamination may begin as early as seeds colonized with human pathogenic bacteria develop into crop plants. The bacteria may spread further via water, either by irrigation or from contaminated manure or soil, or by soil- or airborne vectors (Figure 15.10). Several human pathogenic bacteria, such as the proteobacteria *Pseudomonas aeruginosa* (γ), *Burkholderia cepacia* (β), *Erwinia* spp. (γ), and *Enterococcus faecalis* (*Firmicutes*) amplify on plant tissues. They may inhabit microsites on the plant characterized by the occurrence of sugars and other organic compounds. Competition between regular rhizosphere bacteria and **foodborne pathogens** might be because of preferential growth at root tips and sites of lateral root emergence (sites of solute release) due to easy access to root exudates. *In vitro*, it has been shown that enteric pathogens attach to these sites. Studies using the model plant *Arabidopsis thaliana* (*Brassicales, Rosidae*) have shown, however, that the whole plant can be colonized by, for example, the γ-proteobacteria *Salmonella enterica* and *E. coli* following irrigation with the bacteria. Even flowers and seeds were contaminated, implicating a further problem with spreading pathogenic bacteria. Seedborne outbreaks are frequent with raw sprouts of alfalfa, radish, and mung beens and are also potentially responsible for the recent outbreak of *E. coli* O104:H7 in Germany, resulting in the development of a **hemolytic uremic syndrome (HUS)** in a large group of patients.

Regardless of varying **rhizosphere competence** of human pathogenic and plant-associated bacteria, they have a genetic factor in common that enables both to survive in this niche. The "starvation" sigma factor RpoS involved in *in vitro* stress adaptation in *Salmonella enterica* and *E. coli* also enhances rhizosphere competence in *Pseudomonas fluorescence* and *P. putida*. Sigma factors bind to the bacterial RNA polymerase core complex to form the holoenzyme, thereby providing the ability to recognize promoter sequences on the DNA to initiate transcription (see Section S1.3.10.1). In addition, the presence of cellulose production genes, which are associated with **biofilm** production (see Chapter 14) and of UV radiation-tolerance genes is regarded as a fitness factor for the survival on plant surfaces.

From the genus *Burkholderia* (*Betaproteobacteria*) a total of 62 species have been described. Owing to their clinical importance, species belonging to the *B. cepacia* complex, comprising 17 species isolated from patients with cystic fibrosis, are intensively studied. Isolates have been described, which are able to fix nitrogen and form nodules with legume roots, constituting true endophytes. Interestingly, a phylogenetic analysis of 16S rRNA sequences of all 62 recognized *Burkholderia* species reveals two main clusters: the first one comprises the *B. cepacia* complex, together with other human pathogenic species, plant pathogens, and endosymbionts of pathogenic fungi. The second cluster contains nonpathogenic *Burkholderia* species that are associated with plants and/or the environment. This second cluster has been named "plant-beneficial *Burkholderia* cluster" and contains 31 species.

There is evidence for **horizontal gene transfer** between soil bacteria, which may increase their rhizosphere competence. A major concern is the possible acquisition of antibiotic resistance genes by potential human pathogens on agricultural crops. One other problem is that even

though pathogenic bacteria seem to exist in low numbers on plant material, they seem to be sufficient to cause a high incidence of diseases. This prompted the question whether the plant environment affects the pathogen's physiology in a way that fewer cells are required to cause a disease. One hint in this direction is that plants under microbial stress produce antimicrobial peptides. Such peptides may regulate bacterial determinants that help to overcome the first line in human defense (stomach acid). For example, the operon that confers resistance to host antimicrobial peptides is involved in the virulence in *S. enterica* and is believed to play a role in the resistance of *E. chrysanthemi* to plant antimicrobial peptides in damaged plant tissues. The same operon enables the pathogen to better resist early human host defense.

In light of these reports, bacteria that are suggested as biocontrol agents against microbial plant pathogens need to be studied thoroughly to assess possible risks to humans.

15.2 Fungi of the Rhizosphere

The rhizosphere hosts a great diversity of fungi (*Fungi, Opisthokonta*). They can broadly be separated into the decomposers, plant-beneficial mycorrhizal fungi, and plant pathogenic fungi. **Fungal decomposers** have a saprophytic lifestyle. Dead organic matter is converted into fungal biomass. They are especially important in the degradation of recalcitrant organic matter (lignocellulose), which is degraded by soil bacteria only to a lesser extent. Thus, they play an important role in the recycling of nutrients in the soil. **Mycorrhizal fungi** form a close interrelation with plant roots in which they provide the plant with improved access to water and nutrients, mainly phosphorus and nitrogen. In exchange, the fungi obtain sugars produced by the plant during photosynthesis. It is estimated that over 90% of land plants live in such an association with soil fungi. In aquatic environments, similar groups of fungal colonizers of plant roots can be found. Here, mycorrhizal fungi as well as endophytically growing fungi have been detected, which vary according to the organic matter content and pH of the surroundings. Mostly, the fungal genera involved have been described before as colonizers of terrestrial plant roots with an equally diverse ecology: arbuscular AM as well as ectomycorrhizal ECM fungi (see Section 5.2.1), ericoid and orchid mycorrhizal, and saprotrophs as well as endophytes.

The group of pathogenic soil fungi consists of those that depend on living plant tissues in order to survive. **Pathogenic fungi** are usually not dominant in soils, but infections may increase in managed areas because of the reduction in biodiversity of other soil-inhabiting organisms.

The assessment of a soil fungal community by growing isolates on pure culture media is, however, limited. Fungi of the order *Glomales* (*Glomeromycota*), for example, which are important symbionts ("endomycorrhiza"), can be cultured only in the presence of a host root. The application of molecular techniques, like rRNA-based analyses, largely improves our understanding of rhizospheric fungal communities.

In the following section, the chemical communication between plants and mycorrhizal as well as pathogenic fungi is described.

15.2.1 Mycorrhiza: Chemical Dialogue between Plants and Mycorrhizal Fungi

Most important for plant development and productivity are soil-borne fungi that develop symbiotic structures (**mycorrhiza**). They generally function as more or less mutualistic solute exchange systems. The host plant delivers carbohydrates for fungal growth and maintenance and obtains water and nutrients from the fungus. Among the different types of mycorrhizas, **endo- and ectomycorrhizas** are studied in most detail (see Section 5.2.1).

Development of **AM** is preceded by fungal spore germination, which may result in the retraction of nuclei and cytoplasm in the absence of a plant root. Because flavonoids are key signaling compounds isolated from plant root exudates, they have been proposed to play a distinct role in AM development. Several flavonoids have been shown to affect hyphal growth and differentiation and root colonization in a structure-specific manner. In turn, flavonoids also exert a fungus genus- and species-specific effect during presymbiotic growth. To widen this concept, it has also been suggested that certain soil bacteria that promote the formation of mycorrhiza (so-called **mycorrhiza helper bacteria**, see Section 15.2.3.) act by inducing flavonoid release from plants, thus facilitating root colonization by mycorrhizal plants.

The **strigolactone** 5-deoxystrigol (Figure 15.11, box 1, a) was identified as a signal molecule in the root exudates of *Lotus japonicus* and induces hyphal branching, germination of fungal spores, and alteration of fungal physiology. Because Strigolactones (a group of sesquiterpene lactones) are extremely short lived, they form a distinct gradient around the plant root, indicating to the fungus the proximity of and direction to the root. Other strigolactones, such as strigol, sorgolactone, and a synthetic analogue, GR24, mimic the activity of 5-deoxystrigol. Once the signal is perceived by the fungus, the presymbiotic phase is induced during which hyphal growth and branching and physiological activity is increased. In contrast, the fungal signaling molecules (referred to as **myc factors**) that induce a symbiosis-specific response in the host roots still remain elusive. Myc factors are diffusible molecules that may induce transcriptional changes in a putative host plant and result in enhanced lateral root growth. The involvement of strigolactones in the production of such myc factors is still unknown. Upon contact of the hyphae with the

root, the prepenetration apparatus (PPA) is formed (see Section 5.2.1.1).

Signal molecules in **ECM** formation are much less well known. The involvement of such molecules has been obvious as plant-derived compounds trigger spore germination, hyphal growth toward plant roots, and ECM formation (see Section 5.2.1.2).

Several plant-derived metabolites have been implicated in early events in mycorrhiza formation. Among those are flavonoids, diterpenes, hormones, and nutrients. The phenolic compound **rutin** (Figure 15.11, box1, c), isolated from eucalyptus root exudates has been shown to have a major effect on hyphal growth of certain *Pisolithus tinctorius* strains, whereas a tryptophan derivative, **hypaphorine** (Figure 15.11, box 1, b), which is secreted by *P. tinctorius*, can repress root hair elongation and stimulate formation of lateral roots. Hypaphorine production is increased in the presence of root exudates and during mycorrhiza formation. Antagonism of hypaphorine to plant-produced indole acetic acid (IAA) seems to be involved in the observed short root development. Phytohormones also play an important role in ECM formation. Auxin levels, in particular, play a specific role in ECM development. Plant-derived compounds in the rhizosphere enhance the biosysnthesis of hormones by ECM fungi, resulting in morphological changes and enhanced ECM formation.

15.2.2 Chemical Cross Talk between Plant Roots and Pathogenic Fungi: Signaling Involved in Recognition

Fungi (*Opisthokonta*) and *Oomycetes* (*Stramenopiles*) comprise the most plant pathogens in soil. Because the processes involved in the infection of a plant by such a

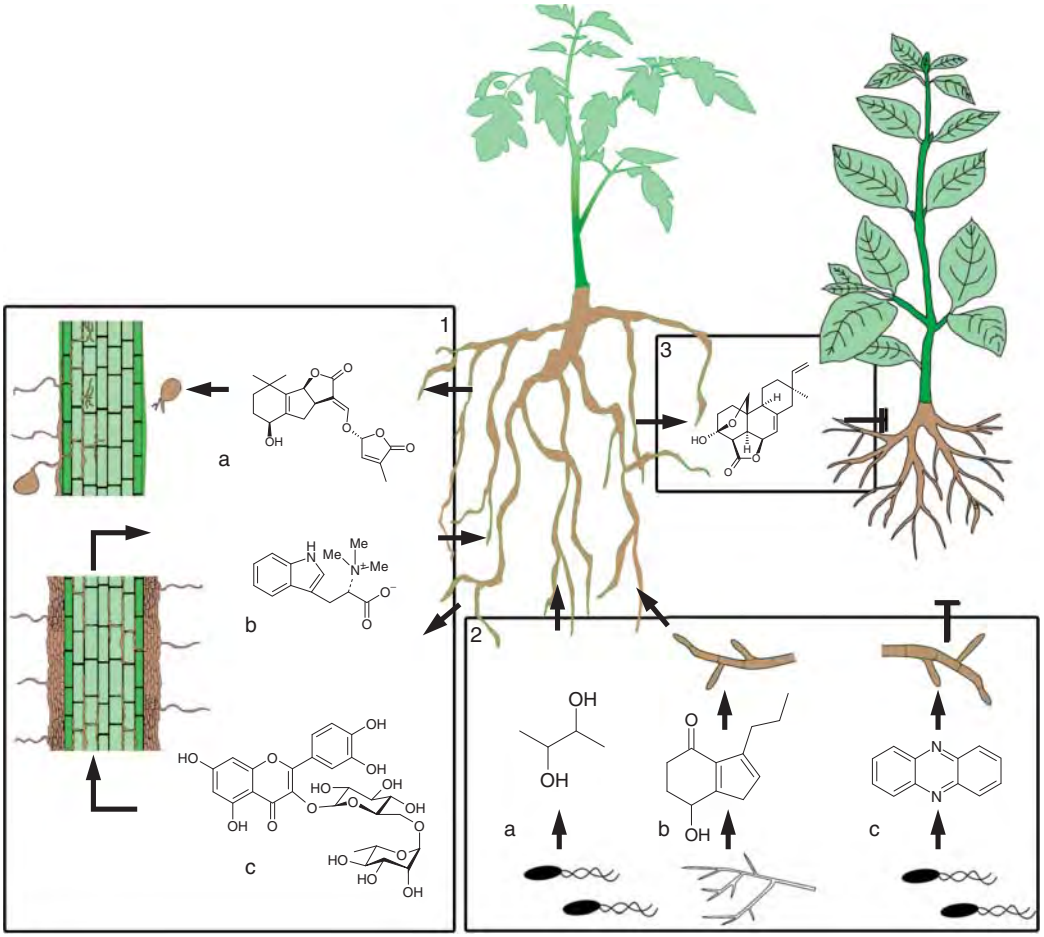


Figure 15.11 Summary of plant interaction with other soil organisms and chemical signals involved. Box 1 highlights chemical signaling in mycorrhiza formation. The strigolactone 5-deoxystrigol (Box 1, a) induces spore germination, hyphal branching, and alteration in fungal physiology in AM fungi. Rutin (Box 1, c), isolated from eucalyptus root exudates, affects the growth of the respective mycorrhizal fungus, whereas the tryptophan derivative, hypaphorine (Box1, b), secreted by the fungus, in turn affects root hair formation and lateral root growth (Section 15.2.1). Box 2 illustrates bacterial effects on plants and on the interaction between plants and fungi. 2,3 butanediol (Box 2, a) improves plant growth (Section 15.1.1). Auxofuran (Box 2, b), isolated from a mycorrhiza helper bacterium, promotes fungal growth (Section 15.2.3.1). Phenazine (c, Section 15.1.2), produced by pseudomonads, has antagonistic activity toward plant pathogenic fungi. Momilactone B (Box 3, Section 15.3.3) is released by rice roots and results in the growth inhibition of neighboring species.

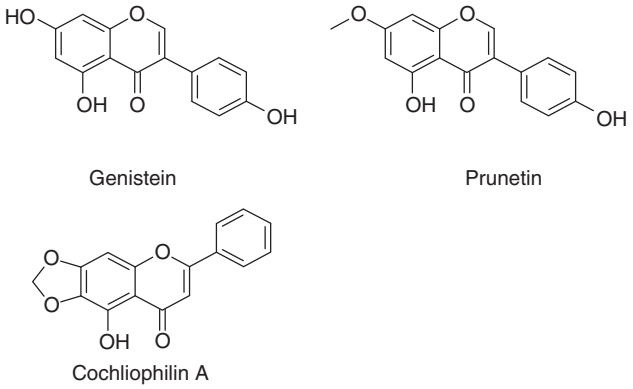


Figure 15.12 Plant-produced attractants for plant pathogenic oomycetes (*Stramenopiles*).

pathogen are very similar to those observed in the formation of arbuscular mycorrhizae (AM) formation, the involved chemical cues are used in several ways. Following contact between hypha and host plant, usually an appressorium is formed where a hyphae penetrates the host tissue. The role of flavonoids has been studied in detail in interactions of plants with Oomycetes. These are fungus-like organisms that are phylogenetically distinct from *Fungi* and contain cellulose within their cell walls as opposed to chitin in true fungi. Oomycetes produce motile zoospores that chemotactically move toward a potential infection site where they accumulate. Following morphological modifications the root tissue is penetrated. To result in an infection, zoospores of *Phytophthora sojae* are attracted by the flavonoids, **daidzein** (Figure 15.7) and **genistein** (Figure 15.12). This effect is host-specific. Zoospores of *Aphanomyces raphani*, for instance, are attracted by the host plant produced substance 3-indolcarbaldehyde, *Aphanomyces euteiches* by **prunetin** (Figure 15.12) and *Aphanomyces cochloides* by **cochliophilin A** (Figure 15.12) at low micromolar or nanomolar concentrations.

In the pathogenic interaction between legume plants and the true fungus *Fusarium* sp. (*Ascomycota, Dikarya*), plant-derived root exudates contain flavonoids that are highly stimulatory toward *Fusarium* spore germination. This stimulation is independent of that induced by nutrients. Whether this stimulation is species specific is still under debate. The specific group of compounds that induces hyphal branching in AM fungi, strigolactones, seems not to play a role in interactions with other fungi. When ECM fungi, plant-beneficial fungi, and plant pathogenic fungi were exposed to GR24 (a synthetic analogue of 5-deoxystrigol (Figure 15.11, box 1, a), no indication of an alteration of hyphal branching pattern could be observed.

15.2.3 Fungus–Bacterium Interactions

Bacteria coexist with fungal hyphae, colonizing surfaces and using compounds exuded from the hyphae. Hyphal exudates consist of low molecular weight metabolites such as complex mixtures of organic acids and iron-complexing siderophores. The bacteria have to be tolerant against antibacterial compounds, produced by the fungus, and even may have developed strategies to increase leakage from colonized fungal hyphae to improve nutrient release. In turn, effects toward the fungal "host" can be observed: bacteria induce a variety of developmental, morphological, and physiological modifications. Hyphal growth and branching, hyphal exudate composition, production of antibacterial metabolites as well as changes in fungal transcriptome have been observed.

Because of the transfer and exudation of plant-derived organic compounds to soil microsites not accessible to roots, mycorrhizal fungi can promote bacterial growth and survival, and soil bacteria can enhance the formation of mycorrhizal structures, either by promoting their establishment and functioning (**mycorrhiza helper bacteria**), or by protecting them from pathogenic microorganisms.

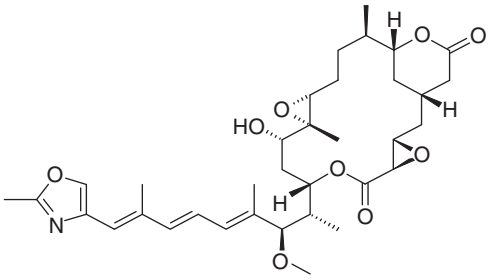
Depending on field conditions, bacteria of the mycorrhizosphere (soil zone influenced by plant root and extending hyphal network) colonize the surface of the fungal hyphae. A comparison of bacterial populations with regard to the host plant and associated (AM-forming) fungi indicate a larger influence of the latter.

With regard to plant nutrition, **bacterial mycophagy** can be of importance. Bacteria have been found to feed on both dead fungal material and surface structures of living fungi (such as the outer layers of spores). The direct antibiosis between bacteria and fungi has been discussed in Section 15.1.2.

In some fungal taxa, bacteria also live inside fungal cells as **endobacteria**. By means of 16S rRNA, endobacteria have been identified in AM-forming fungi. A model system of such an interaction has recently been developed with an isolate of the fungus *Gigaspora margarita* (*Glomeromycota*) and its β-proteobacterial endobacterium "Candidatus *Glomeribacter gigasporum,*" which is now investigated at the molecular level.

Another example is a well-investigated member of the fungal order of the *Sebacinales* (*Basidiomycota, Dikarya*), *Piriformospora indica*. This basidiomycete promotes growth and vitality of a large range of plant species, even of members of the Brassicaceae (*Brassicales, Rosidae*), which do not form mycorrhiza-like symbioses. On the basis of 16S rRNA analysis, rod-shaped bacteria detected in the cytosol of the fungal cells have been shown to be related to the α-proteobacterium *A. tumefaciens* (*Rhizobium radiobacter*). The bacterium can be kept in single culture and also shows plant growth promoting properties like its host fungus. In contrast to *A. tumefaciens*, it lacks pathogenic effects at low cell numbers. Probably there is some control of pathogenicity by the fungal host.

The complexity of such fungus–endobacterium association becomes obvious in the interaction between the rice seedling blight-causing agent *Rhizopus* (a zygomycete,



Rhizoxin

Figure 15.13 Rhizoxin, synthesized by a bacterial endophyt of the genus *Burkholderia* (*Betaproteobacteria*) of the rice pathogenic fungus *Rhizopus* sp. (*Fungi, Opisthokonta*).

Mucoromycotina) and its endofungal β-proteobacterium of the genus *Burkholderia.* The macrocyclic polyketide metabolite **rhizoxin** (Figure 15.13) that causes an abnormal swelling of the seedling roots is not produced by the fungus itself but by the endosymbiotic bacterium. Research about the interaction between rhizosphere fungi and endobacteria is still at the beginning, but opens another highly interesting aspect of rhizosphere interactions.

15.2.3.1 Mycorrhiza Helper Bacteria

Many bacterial isolates have been shown to exert a positive effect either on the formation of mycorrhiza or on the functioning of the mycorrhizal complex. Such **mycorrhiza helper bacteria** have been reported from Proteobacteria (*Agrobacterium, Azospirillum*, *Azotobacter*, *Burkholderia*, *Bradyrhizobium*, *Enterobacter*, *Pseudomonas, Klebsiella*, *Rhizobium*), Firmicutes (*Bacillus*, *Brevibacillus*, *Paenibacillus*) and Actinobacteria (*Rhodococcus*, *Streptomyces*, *Arthrobacter*). Mechanisms behind the promotion of mycorrhiza formation can broadly be separated into: (i) affecting the fungal partner (mycelial growth, spore germination); (ii) modifying the host root architecture (increase of root fungus contact sites by increasing the number of lateral roots; and (iii) controlling of soil properties (nutrient mobilization, metabolization of compounds inhibitory to mycelial growth). Numerous examples exist that show the promotion of fungal spore germination and hyphal extension of mycorrhizal fungi by mycorrhiza helper bacteria. The active substances can also be volatile and be released to the gas phase as has been shown with streptomycetes that stimulate AM fungal spore germination. The improved mycorrhization is thus often suggested to be a result of the increased potential contact areas for fungi and plant roots. In the interaction between the mycorrhizal fungus fly agaric (*Amanita muscaria, Basidiomycota, Dikarya*) and a mycorrhiza helper bacterium belonging to the genus *Streptomyces*, enhanced mycelium extension was observed due to the bacterial secondary metabolite **auxofuran** (Figures 15.8 and 15.11, box 2, b). This compound was produced by a streptomycete in single culture but even more so in dual culture with the mycorrhizal fungus itself.

An influence of the mycorrhiza helper bacteria toward potential host plants has also been described. Frequently, the increase in lateral root formation in the presence of such bacteria is observed. In combination with improved mycelial growth, this results in potential interaction points between plant root and fungus. The plant hormones auxin and ethylene are suggested to play a role in such modifications of the root system, yet this has not been connected to the production of such hormones by mycorrhiza helper bacteria.

15.2.3.2 Bacterial Mycophagy

Bacterial mycophagy describes the potential of bacteria to grow on living fungi and convert the released nutrients into bacterial biomass. In necrotrophic interactions, cell death of fungal hyphae results from the release of bacterial toxins, proteins, or cell wall degrading enzymes. Following the lysis of cells or the inhibition of fungal metabolism (i.e., killing of fungal cell), nutrients released are ingested by bacteria (Figure 15.14). Examples are numerous and can be found among the *Actinobacteria*, *Betaproteobacteria,* and *Firmicutes* bacteria of the genus *Bacillus*. Chitinase activity is commonly observed among those bacteria, yet the fungal cell wall is a difficult target comprising various polysaccharide and protein components that require a vast array of cell wall degrading enzymes for breakdown. Chitinase activity alone is thus not sufficient for the effective killing of hyphae. Instead, bacterial toxins seem to be involved. The lipodepsipeptide tolaasin produced by mushroom-infecting *Pseudomonas* isolates acts at different targets. It induces pore formation in plasma membranes and has a strong surfactant activity, reducing hydrophobicity of the hyphal surface. Possibly, cell wall degrading enzymes act in the next step to successfully lyse hyphal cells.

15.3 Plant–Plant Interactions

Plant roots do not exist solitarily under natural conditions. They are either in intimate contact with other eu- and prokaryotic organisms or are connected to them, for example, through fungal hyphal networks. This illustrates that communication between plants via neighboring roots will not be merely bidirectional (Section 15.3.1). A direct interaction of plants resulting in the **parasitism** of one plant on the other is brought about by the same chemical cues that aid in the development of AMF symbiosis, indicating the abuse of such signals for parasitic purposes (Section 15.3.2). A direct interaction without the need for physical contact can be observed in case of **allelopathy**, the production of chemicals acting as spacers to keep away the invading neighbors (Section 15.3.3).

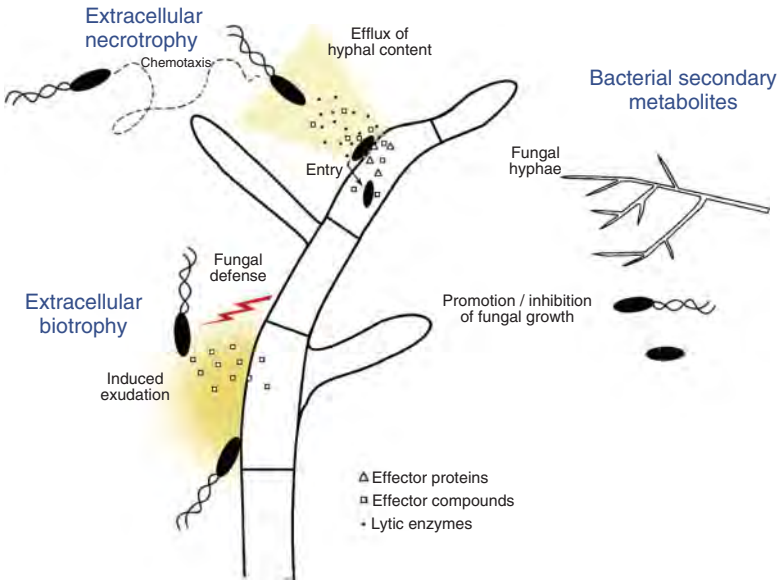


Figure 15.14 Fungus–bacterium interactions: during extracellular necrotrophy, bacteria lyse and kill hyphal cells by producing toxins and lytic enzymes. In extracellular biotrophy, bacteria make use of fungal exudates and thus need to be tolerant against antibacterial metabolites secreted by the fungus. Filamentous bacteria like Streptomycetes (top right) and other bacteria (below) are prolific producers of fungal growth modulating secondary metabolites. (Adapted from Leveau and Preston (2008).)

15.3.1 Plant–Plant Interaction via Fungal Networks

Hyphal strands connect neighboring trees and can establish a large network (common mycorrhizal network, or wood wide web, www) for assimilate transfer according to source (net producer)–sink (net consumer of assimilates) relationships. Net translocations of carbon, nitrogen, and phosphorus between plants connected by such hyphal networks have been described. **Common mycorrhizal networks** may form between the same or different plant species and also exist between fine roots of seedlings and of adult trees, possibly helping to compensate for shadowing and thus increasing seedling performance under light limitations. Molecular proof for such networks comes from DNA analysis of roots and associated fungi. *Rhizopogon* (*Basidiomycota, Dikarya*) mycorrhizas, for example, were found to colonize up to 19 trees in one plot. Although ECM is typical for trees and shrubs, some herbaceous plants as well form this type of mycorrhiza. The latter have possibly an important function in bridging forest gaps by spreading ECM fungi, as well as perpetuating fungal inocula when, for example, after fire, tree seedlings start to reestablish.

Endomycorrhiza common mycorrhizal networks have been shown to have a strong positive effect on the transfer of pathogen resistance to the host plant, e.g. in tomato plants mycorrhized with *Glomus mosseae* (*Glomeromycota*) and partly infected with the pathogenic fungus *Alternaria solani* (*Ascomycota, Dikarya*). In due course, the receiving (uninfected) plant showed increases in disease resistance and activities of potential **defense-related enzymes** such as peroxidase, polyphenol oxidase, chitinase, ß-1,3-glucanase, phenylalanine ammonia-lyase, and lipoxygenase and upregulated expression of six defense-related genes. Thus, plants may communicate via this underground communication conduit to transfer signals that induce pathogen defense.

Another aspect for which networking might be important is the **water exchange** between plant and fungus. The role of common mycorrhizal networks in the distribution of water between plants is not resolved. Because water movement is always a passive process along a water potential gradient, environmental conditions determine whether a mycorrhizal fungus can add to the plant's water supply. It was suggested that mycorrhiza help plant survival during transition from moist to dry conditions by being able to explore water reservoirs in soil pores; however, in this process, the flow of water from plant to fungus has also been observed. The **hydraulic lift (HL)** has been shown to help maintain the integrity of mycorrhizal mycelia during drought conditions. There is also evidence that water is translocated from plants performing HL through mycorrhizal hyphae to neighboring plants. This indicates that the water status of plants sharing a common mycorrhizal network might be maintained by one plant doing the HL; from there it could be shared during drought, at least as long as mycorrhizal linkages are maintained.

15.3.2 Parasitic Plants

Lifestyles of parasitic plants vary greatly across angiosperm taxa, and studies have shown that parasitism originated several times independently. Best-studied examples of parasitic plants that infect their host via their roots are ***Striga***

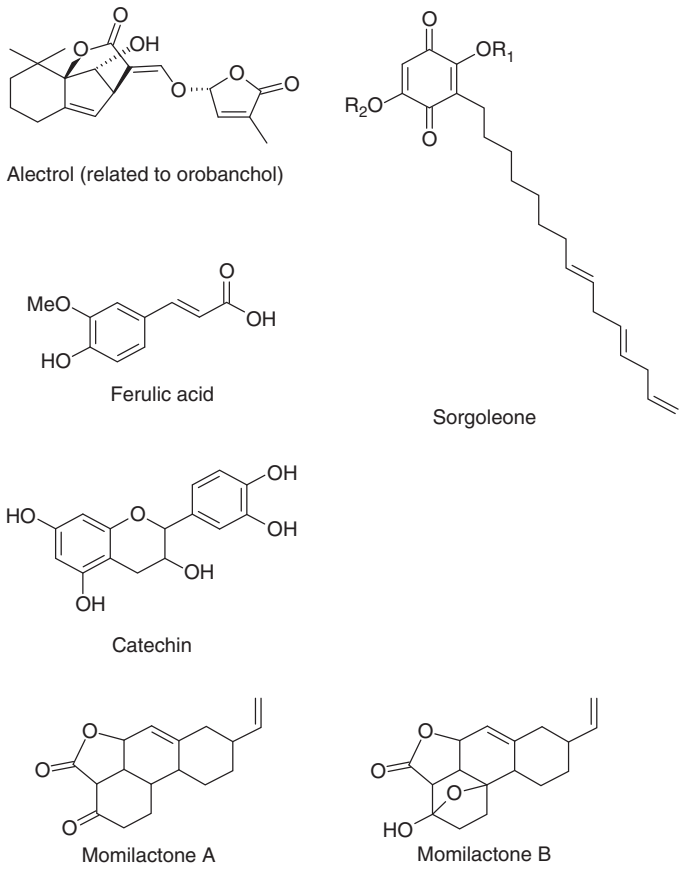


Figure 15.15 Chemicals involved in plant–plant interaction (Allelochemicals).

and ***Orobanche*** (both *Lamiales, Asteridae).* Seedlings of both survive only briefly following germination and they germinate only in the presence of chemical cues. Strigolactones (sesquiterpene lactones, see Section 15.2.1.) are released by plant roots. They are unstable in soil and degrade quickly and induce a germination signal only within some mm distance from the root. It is assumed that a gradient in strigolactone concentration directs the growth of the emerging parasite radical toward the host root. The first such substance, strigol, was isolated from the nonhost cotton, and subsequently several true host plants (maize and millet) were found to also produce strigol. Structurally similar strigolactones were isolated from sorghum, red clover (**alectrol**, Figure 15.15), and from *Lotus japonicus* (5-desoxy-strigol, Figure 15.11, box 1, a, *Fabales, Rosidae*).

How exactly strigolactones trigger seed germination is not well understood. Several factors seem to be important: gibberellin synthesis has to be concomitant in seeds and ethylene triggers seed germination of *Striga* and *Orobanche*. Most interestingly, strigolactones also serve as important triggers in AM development (see also Section 15.2.1 and Figure 15.11), suggesting that parasitic plants may have co-opted these signals to recognize and locate a potential host plant. Following germination, such compounds result in the formation of haustoria. In addition, hydrogen peroxide is constitutively released from *Striga* germlings into the rhizosphere where it activates host peroxidases and degrades host cell wall pectins. In due course, benzoquinones are then released into the rhizosphere. These also result in haustorium formation by *Striga* roots. Studies on the molecular background indicate that benzoquinones downregulate a gene coding for one *Striga* expansin protein, while two other expansin genes are upregulated. Because expansin functions by releasing hydrogen bonds in cell walls, this regulation may play an important role in *Striga* haustorium formation.

15.3.3 Allelopathy

A distinct form of plant–plant interaction, in which one plant specifically interacts with the neighboring one, is called *allelopathy*. The expression "allelopathy" was first defined by the Austrian plant physiologist Hans Molischeme in 1937 to describe the observation that plants are able to banish surrounding plants of the same or different species. Over time, this definition was extended and now includes not only the effects of plants on other plants but also on microorganisms, which is actually a definition of chemical ecology in general.

A comparison of the chemical identities of **allelochemicals** shows that the biochemical origin cannot be

implemented to determine the "assignment" of a substance, as the production and release of allelochemicals are the result of general plant behavior impacting almost all aspects of plant ecology. This is problematic because some substances, for example, siderophores, can act as a nutritional factor but also as an allelochemical. **Ferulic acid** (Figure 15.15) is a siderophore but its action toward other organisms follows the hormesis effect (low concentrations can be favorable, higher ones toxic). Which properties does a substance need to qualify as an allelochemical? Studies have primarily focused on the phytotoxic action while other important features, such as the mode of release, bioactive concentration, persistence, and fate in the environment, are less commonly analyzed. A further problem of phytotoxic activity is that lowest doses that inhibit plant growth in field studies are a magnitude higher than what should be expected under natural conditions.

The production of allelochemicals may be constitutive or induced by biotic or abiotic factors present in the ecosystems in which plants grow. In turn, chemicals produced by plants have strong effects on **ecosystem properties**. Passage from the plant into the rhizosphere can be either passive by diffusion or by directed transport via vesicles and transporters and results in the next problem with the study of allelopathy: once the potential allelochemical has left the root, it is subject to soil physical properties that might result in oxidation or immobilization by binding to soil particles or to degradation or metabolic turnover by microorganisms.

To focus on these problems, the aforementioned ferulic acid and the vast amount of phenolic compounds serve as good examples. **Phenolics** (consisting of a hydroxyl group attached to an aromatic hydrocarbon) are abundantly produced by plants and can be found in plant decomposition products. Their biological activity ranges from changing membrane permeability, inhibiting plant nutrient uptake, affecting plant photosynthesis and respiration, and modifying protein synthesis and enzyme functioning. However, in soil they can exist either in free or bound forms. Depending on the availability, such compounds may alter their activity toward target organisms, resulting in the described effects or in its ineffectiveness.

The influential effect of allelopathic compounds on microorganisms leads to indirect effects on competing plants. For example, allelochemicals that negatively affect the growth of mycorrhizal fungi may serve as an advantage for nonmycorrhizal plants. Plant-beneficial bacteria can also be the target of such substances. The ability to inhibit symbiotic rhizobia strains would provide the producing plants with an advantage over a plant that depends on the symbiotic interaction. Further, microbial transformation of such chemicals plays a critical role in the outcome of the allelopathic effect. Microbial degradation of compounds may result in a detoxification thus disenabling a negative effect; it may also render a substance even more allelopathic.

Regardless of these difficulties, a well-studied example is the production of **momilactone** as an allelochemical released by rice roots. A large number of rice varieties have been found to inhibit the growth of neighboring species under field conditions as well as in the laboratory. In the search for allelochemicals responsible, momilactone A and B were identified (Figure 15.15). Both had been isolated from rice husks in the 1970s. In rice plants (*Oryza, Poales, Liliopsida*), momilactones are synthesized as part of a defense responses resulting in antibacterial and antifungal activities in leaves. In leaves, momilactone A and B exert antifungal activity toward the pathogenic rice blast fungus *Magnaporthe oryzae* (*Ascomycota, Dikarya*), which is the most serious fungal disease in rice, capable of causing considerable losses in rice crop yield. Inoculation of *M. oryzae* to rice leaves rapidly increased the expression of momilactone biosynthesis genes and subsequent accumulation of momilactone A in the leaves. As a result, fungal DNA in the leaves decreased.

Allelochemicals also have been suggested to play important roles in the distribution of **invasive plant species**. The spotted knapwood (*Centaurea stoebe, Asterales, Asteridae*) was introduced to North America around 100 years ago. In its natural habitats, Eastern Europe and Asia, *Centaurea* sp. is a moderately sized plant and a minor component of plant communities while it grows aggressively in North America where it dislodges the naturally occurring flora, indicating the involvement of allelochemicals. The main compound produced by *Centaurea* sp. is a racemic mixture of +/− **catechin** (a flavan-3-ol (Figure 15.15)). Catechin has been detected not only *in vitro*, but also in natural soils. Furthermore, it has a stronger effect on native North American species compared to its long-term European neighbors. These findings suggest that the strong allelopathic effects caused by catechin play a role in the weed's invasive success. Catechin also affects soil and root zone microbial communities. Native systems (including bacterial communities) adapt to allelochemicals such as catechin over time. Interestingly, when the compound was tested on isolated bacterial strains, a reversible bacteriostatic effect was observed, and removal of catechin from the system allowed normal bacterial growth kinetics to resume. This suggests that bacterial communities exposed to allelochemicals in soil might recover to their native structure and function on the removal of such compounds by degradation or transformation. This observation shows that adaptation of an invasive plant species or microorganism community to the chemistry of other species appears to be crucial to the organization and development of a community. The invading plants use compounds novel to the new environment to their advantage in the interaction with the native plants and microorganisms. Soil microorganisms that are able to detoxify specific chemicals probably evolved to do so. This could further lead to an increase in the biological activity of a thus far unknown compound that will not easily be degraded because of accumulation and prolonged persistence in the soil.

Sorgoleone, a major component of *Sorghum bicolor* (*Poales, Liliopsida*) root exudates, is another well-studied allelochemical (Figure 15.15). Again, the ability of *Sorghum* to cause "**soil sickness**" and inhibit the growth of other crops has been known for a long time. Exudation from root hairs does not exceed 20 $\mu g\, mg^{-1}$ root dry weight and it seems to be subject to a feedback inhibition mechanism, because following exudate removal, production continues. The nature of the regulation of the production is still unknown, but because it is obviously controlled by a feedback mechanism, it appears to depend on the accumulation of either sorgoleone or one of its intermediates. This could be a way to avoid autotoxicity.

Regarding the phytotoxic capabilities of sorgoleone, small seeded plants are much more prone to inhibition than large seeded ones. Large seeded weed might be less sensitive to sorgoleone by having lower absorption and translocation, faster metabolic degradation, or by having more rapid initial root growth beyond the location of *Sorghum* root exudates. Sorgoleone enters the soil directly via the root hairs and, as this happens continuously, it may be an advantage over other soil applied herbicides, possibly resulting in a greater persistence because of sustained production, as well as continuous distribution according to root growth.

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