Chapter 16
Ectomycorrhiza and Secondary Metabolites

Hanna Dahm and Patrycja Golińska

16.1 Introduction

Approximately 6,000 species of ectomycorrhizal (EM) fungi have been described, considerably more than arbuscular (AM) fungi. This has led to assumption that EM fungi are most host specific than AM fungi. Host plant diversity, species composition, and age do have a role in regulation mycorrhizal communities. Some studies also suggest that plant secondary metabolites (SM) which are in part under plant genetic control can affect EM colonization.

The main function of SM is defense against herbivores and microbes; some SM are signal and attract compounds for seed dispersing animals and some play a role in the symbiotic relationships with plants and microorganisms.

Early in the twentieth century, it was considered that SM arise either spontaneously or with the aid of nonspecific enzymes. Now, there is good evidence that biosynthetic enzymes are highly specific. As a consequence of specific enzymatic synthesis, final products always have a distinct stereochemistry (Wink 2008). Only the enzymes that are involved in the degradation of SM (glucosidases, esterases, and other hydrolases) are less substrate specific.

SM are not functionless waste products, but are important substances for the symbiotic organisms. Precursor for SM synthesis usually derive from basic metabolic pathways such as glycolytic, Krebs cycle, and shikimate pathway. These bioprocesses may lead to synthesis of glucosinates, cyanogenic glucosides, alkaloids, nonprotein aminoacids, amines, flavonoids, terpenes, quinoline, indole, pyrrolidine, pyrrolizidine, alkaloids, cumarins, mono-, sesqui-, and triterpenes.

Some of the genes that encode biosynthetic enzymes have already been isolated and characterized. Wink (2008) consider question of when, where, and how the plant genes evolved that encode enzymes of SM biosynthesis, as well as those of transport, storage, and turnover.

H. Dahm (✉) and P. Golińska
Faculty of Biology and Earth Sciences, Department of Microbiology, Institute of General and Molecular Biology, University of Nicolaus Copernicus, 87-100 Toruń Gagarina 9, Poland
e-mail: dahm@biol.uni.torun.pl

Theoretically some scenarios can be considered:

- Secondary metabolism could be a young phenomenon and modern plants have developed their pathways independently.
- Alternatively, secondary metabolism is an old innovation, which was developed early in the evolution of land plants and was inherited by modern plants.
- Plants could have developed the genes of SM from their own genes of primary metabolism. Starting with duplication of a gene, the new gene became mutated, exhibited new metabolic functions and was established by natural selection.
- Plant might have inherited some of the genes in early evolution by horizontal gene transfer from their bacterial symbionts, which later developed into modern mitochondria and plastids. Bacteria, especially *Actinomyces*, *Streptomyces*, cyanobacteria produce a wide diversity of SM, showing similar structures as plant SM (antraquinones, terpenoids, and alkaloids).

About 80% of modern plants live in symbiosis with fungi (endo-, ectomycorhiza). These fungi could directly have supplied its host with SM or might have transferred (horizontal) the genes to the host's genome.

Environmental factors (biotic and abiotic) control and regulate the biosynthesis of SM in plants (Laitinen et al. 2005; Zhi-lin et al. 2007).

As a defense reaction plants evolved bioactive compounds, which repel, deter, or poison herbivores and which can inhibit growth and development of bacteria, fungi, and viruses. Some of the defense compounds are constitutive, while others can be induced under stress conditions. Several SM (phytoalexins) and defense proteins are synthesized de novo when plant is invaded by microorganisms (Wink 2008).

Type of SM	Estimated numbers[a]
Nitrogen containing SM	
Alkaloids	21,000
Nonprotein amino acids (NPAAs)	700
Amines	100
Cyanogenic glycosides	60
Glucosinolates	100
Alkylamides	150
Lectins, peptides, polypeptides	2,000
SM without nitrogen	
Monoterpenes (C10)[b]	2,500
Sesquiterpenes (C15)[b]	5,000
Diterpenes (C20)[b]	2,500
Triterpenes, steroids, saponins (C30, C27)[b]	5,000
Tetraterpenes (C40)[b]	500
Flavonoids, tannins	5,000
Phenylpropanoids, lignin, coumarins, lignans	2,000
Polyacetylenes, fatty acids, waxes	1,500
Polyketides	750
Carbohydrates, simple acids	400

[a]Approximate number of known structures
[b]Total number of all terpenoides exceeds 22,000 at present

In the plant–microbe interaction, coevolution between plants and their microbial partners are mediated via plant chemical defense. Plant SM usually act as signal molecules or respond to pathogen and symbiont colonization. Mycorrhizal associations are the most important mutualist symbiosis which involve three-way interactions between plants, mycorrhizal fungi, and soil factors. Interactions in the mycorrhizal associations between macro- and microsymbiont in contrast to plant–pathogen interactions are for both profitable.

In presymbiotic phase, plant and their fungal partner secret signals into soil, mostly SM, recognized by roots and mycelium, inducing morphological and physiological modifications.

According to some investigators (Baron and Zambryski 1995; Garcia-Garrido and Ocampo 2002) signal perception and transductions proceed via similar pathways between symbiosis and pathogenesis of plants. However, the defense response in plant-mycorrhizal is probably weak.

The nature of signaling molecules, signal perception, and transduction in mycorrhiza are unknown or mistakenly denied (Martin et al. 2001). In the first stage, host plants release into the rhizosphere metabolites that are able to trigger basidiospore germination, growth of hyphae toward the roots and the early steps of mycorrhizal formation.

According to Kottke and Oberwinkler (1987), Horan et al. (1988), Lagrange et al. (2001), Martin et al. (2001), molecules that control the interactions between symbionts can be classified as follows:

- Tropism of hyphae for root tissues (rhizospheric signals)
- Attachment and penetration of host tissues by hyphae (adhesions, hydrolases)
- Induction of organogenetic programs in both fungal and root cells (hormones and secondary signals)
- Facilitating survival of the mycobiont despite plant defense responses
- Coordinating strategies for exchanging carbon and other metabolites for plant

Signals secreted into the rhizosphere can include flavonoids, terpenes, hormones, and various nutrients. These substances stimulate growth and modified hyphal morphology.

Some of these substances might be produced and released into rhizosphere by bacteria, namely mycorrhization helper bacteria (MHBs). Root exudates enhanced accumulation of fungal molecules such as hypaphorine, the betaine of tryptophan (Martin et al. 2001). This fungal alkaloid is the major indole compound produced in larger amounts by some EM fungi (e.g., *Pisolithus* sp.) during mycorrhiza formation and development (Béguiristain and Lapeyrie 1997; Martin et al. 2001).

Hypaphorine induces morphological changes in root hairs, which lead to a decreased rate of elongation and transitory swelling of the apex of the root hair (Ditengou et al. 2000).

Root hairs are a significant site for microbial interaction in the rhizosphere and it has been suggested that interaction between the EM fungus and root hairs may play a role in the symbiosis development. Growth in root hairs is associated with an apex-high cytosolic free Ca^{2+} gradient generated by a local Ca^{2+} influx at the tip.

Some investigators suggest that hypaphorine-induced cytoskeleton changes are related to interaction with calcium channels, cofilin/actin depolymerizing proteins and auxin signaling pathways (Ditengou et al. 2000).

The aim of this chapter was to point out a presence of various SM released both the micro- and macrosymbiont to the mutual interactions zone.

Any such chemical compounds (both the volatile and nonvolatile ones) affect either positively or negatively the mycorrhiza symbiosis formation and functioning as well as such chemical compounds interact between themselves. The role of some metabolites in these processes is better known (auxins), however any importance of the majority of them is still unknown and requires future, detailed studies.

16.2   Flavonoids

Flavonoids are derived from y-pyrone. They are either 2-phenylbenzopyrone or 3-phenylbenzopyrone. More than 1,300 different flavonoid compounds have been isolated from plants. Individual flavonoids in a group differ from each other by the number and position of the hydroxyl, methoxy, and sugar substituents.
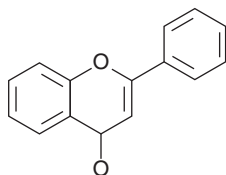
2-Phenyl-1,4-benzopyrone

Flavonoid compounds occur in plants as glycosides, with hexoses such as glucose, galactose and rhamnose, and pentoses such as arabinose and xylose as the most commonly found sugars. The sugars can be attached singly or in combination with each other.

Flavonoids are synthesized by the phenylpropanoid metabolic pathway in which the amino acid phenylalanine is used to produce 4-coumaroyl-CoA. This can be combined with malonyl-CoA to yield of compounds called chalcones, which contain two phenyl rings. Conjugate ring-closure of chalcones results in the familiar form of flavonoids, the three-ringed structure of a flavone.

Flavonoids form a large and heterogeneous group of SM having a bioactive role in the major processes of plants (e.g., attraction of seed disperses, defense reaction against predators, pathogens and abiotic stress condition [Taylor and Grotewold 2005; Niemi et al. 2007]).

Some authors suggest the role of flavonoids in modulating cell signaling pathways including polar auxin transport (Brown et al. 2001; Peer et al. 2004; Kakiuchi et al. 2006).

Despite increasing evidence the role of flavonoids and other phenolic substances in plant development, their role in EM symbioses is contradictory. Studies on the changes in the concentrations flavonoids in Scots pine seedlings during the

establishment of the EM symbiosis with *Suillus variegatus* showed that in contrast to shoots, the concentrations of catechin and condensed tannins showed a tendency to decrease in the roots of both noninoculated and inoculated seedlings and regardless of high mycorrhiza frequencies the fungi caused hardly changes in the flavonoid concentrations of the roots.

Weiss et al. (1997, 1999) suggested that catechin and epicatechin accumulated in the inner part of the cortex to prevent the growth of the EM fungus into the inner cortex.
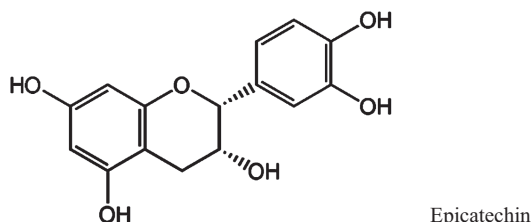
Epicatechin

In contrast, Beyler and Heyser (1997) reported that reduction of catechin and epicatechin in the root tips is a prerequisite for rapid mycorrhization. Similarly, Schützendübel and Polle (2004) showed that Scots pine short root tips covered by the mycelium of *Pisolithus tinctorius* contained less catechin than nonmycorrhizal ones.

16.3 Terpenes

Terpenes are widespread in nature, mainly in plants as constituents of essential oils. Many terpenes are hydrocarbons, but oxygen-containing compounds such as alcohols, aldehydes, or ketones (terpenoids) are also found. Their building block is the hydrocarbon isoprene, $CH_2 = C(CH_3)–CH = CH_2$.

Terpene hydrocarbons are classified according to the number of isoprene units: monoterpenes (2 isoprene units [i.u.]), sesquiterpenes (3 i.u.), diterpenes (4 i.u.), triterpenes (6 i.u.), tetraterpenes (8 i.u.). Examples of monoterpenes are: pinene, nerol, citral, camphor, menthol. Examples of sesquiterpenes are: nerolidol, farnesol. Examples of diterpenes are: phytol, vitamin A_1. Squalene is an example of a triterpene and carotene is a tetraterpene.

When terpenes are modified chemically, such as by oxidation or rearrangement of the carbon skeleton, the resulting compounds are generally referred to as terpenoids. Terpenoids are also known as isoprenoids.

Terpenes are produced by a wide variety of plants, particularly conifers. They are the major components of resin and of terpentine produced from resin. In conifers, biosynthesis and infiltration of tissues with resins are involved in defense system in response to wounding and subsequent inhabitation of the wounds by fungi and insects (Higuchi 1985; Werner 1993; Napierała-Filipiak et al. 2002). The response is nonspecific and similar after wounding and infection.

Members of Pinaceae produce two types of resins – oleoresin and parenchyma resins. Oleoresin is a super saturated solution of resin acids in liquid terpenes and is located in resin ducts and surrounding epithelial cells. Parenchyma or medullary resins are composed mainly of fatty acids (Prior 1976; Napierała-Filipiak et al. 2002).

Pine oleoresin contain 60–70% resin acids. The rest is comprised of volatile and other terpenoids. Among monoterpens, α-pinene, Δ^3-carene, and β-pinene dominate in the volatile fraction (Asiegbu et al. 1998).

The monoterpenes are toxic to wood-fungi, whereas the resin acids display low toxicity and function mainly as mechanical barrier (Prior 1976; Napierała-Filipiak et al. 2002). According to Mekin and Krupa (1971) and Krupa et al. (1973) volatile substances may play a role in the initiation and development of the mycorrhizal symbiosis, because colonization of pine roots by different mycorrhizal fungi results in quantitative changes in the concentration of the individual volatiles.

Phenolics and volatiles are significant factor root exudates and influence the activity of the rhizosphere microorganisms. Due to their volatility, the monoterpenes and some sesquiterpenes can display significant effect on fungi composition structure (Smith 1987). They can regulate competitive or antagonistic interactions and finally create environment stimulating the symbiotic associations.

Forest litter reach in volatile substances may be too a significant factor in the interactions between microorganisms in soil and rhizosphere. Koide et al. (1998) found that á-pinene and â-pinene showed differential effects on the growth of various EM fungi.

Varese et al. (1996) observed enhanced fungal growth due to volatiles; however, the stimulation was seldom significant as some of the substances, when present in sufficient concentration, may cause inhibition of the vegetative growth of mycorrhizal and pathogenic fungi outside the roots.

Differences in the sensitivity of EM fungi to several volatile substances might characterize of their ability to induce host reaction and consequently the ability to initiate symbiosis (Mekin and Krupa 1971; Napierała-Filipiak et al. 2002). Increased production of volatile and nonvolatile substances might be a mechanism of control over growth of mycorrhizal fungi in tissues of macrosymbiont (Molina and Trappe 1982).

Although the major volatiles identified in nonmycorrhizal and mycorrhizal roots of pine, the degree of accumulation of several compounds varied among fungal treatments. This suggests that each mycorrhizal fungus may elicit a different response in trees (Napierała-Filipiak et al. 2002). Also in the previous study by Krupa et al. (1973), the diverse levels of Δ^3-carene and β-phellandrene in roots of *Pinus echinata* inoculated with *P. tinctorius* and *Cenococcum graniforme* were explained in terms of the different ability to elicit a specific host response of the two fungi.

16.4 Plant Growth Regulating Substances (phytohormones)

It is assumed that hormones of plant and fungal origin may take part information and functioning of mycorrhizae (Gogala 1991). Phytohormones, SM synthesis by plant and EM fungi include auxins, cytokinins, GAs, abscisic acid (ABA), ethylene as well

as alkaloids and phenylglycoside (Gogala 1991). The soil pool of phytohormones might have partially originated from plants released into the rhizosphere as root exudates and/or synthesized by soil microorganisms. These biomolecules respond to exchange of rhizospheric signals between microorganisms and plants. Signal perception may culminate in the induction of down-stream target gene products whose expressions are physiological and/or development responses (Martin et al. 2001).

16.4.1 Auxins

Several naturally occurring auxins include indole-3-acetic acid (IAA), its halogenated derivatives (4-Cl-IAA), and indole-3 butyric acid (IBA). On molecular level, auxins have an aromatic ring and a carboxylic acid group.

Auxins play an essential role in coordination of many growth and behavioral processes in the plant life cycle. They act in concert with (or opposition) other plant hormones. For example, the ratio of auxin to cytokinin in certain plant tissues determines initiation of root vs. shoot buds.

The plant hormones stimulate cell elongation. Auxin induces new root formation by breaking root apical dominance induced by cytokinins. However, high concentration of auxin inhibits root elongation and instead enhances adventitious root formation. In low concentration, auxin can inhibit ethylene formation and transport of precursors in plant; however, high concentration of auxin can induce the synthesis of ethylene.

Auxin production is widespread among many mycorrhizal fungi (Gay 1986; Gay and Debaud 1987; Frankenberger and Poth 1987; Kampert and Strzelczyk 1989).

Several studies have demonstrated increased auxin content (hyperauxiny) in response to mycorrhizal infection, which may indicate a role of auxin in EM symbiosis. Studies initiated by Slankis (1950) had shown that auxins as well as cytokinins are necessary for the formation of mycorrhizal structures.

Auxins added to the synthetic media inhibited elongation of pine seedlings roots. The roots became thicker and dichotomically branched devoid of roots hairs and caps, structures characteristic for the nonmycorrhizal roots.

IAA
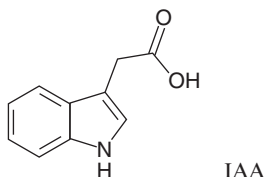
Many studies indicate that changes in auxin balance are a prerequisite for mycorrhiza organogenesis (Gay et al. 1994; Martin et al. 2001). EM fungi enhance proliferation of short roots and the presence of plant-derived tryptophan in the root exudates could be sufficient for EM fungi to enhance the biosynthesis of fungal IAA (Rupp et al. 1989).

Although the structure of ectomycorrhizae in a natural habitat may show considerable variation, common features are a swollen appearance, lack of root hairs, and variable radial growth of cortical cells within the swollen region (Slankis 1973).

The fact that roots are very sensitive to auxins and that auxins take part in many physiological and metabolic processes can be expected that the presence of excess auxin in mycorrhizae would profoundly affect their physiology and metabolism. EM roots morphology reflect a specific physiological and metabolic state which is necessary for the functioning of the symbiosis.

According to Slankis (1973), the hyperauxiny in mycorrhizal roots is more likely to result from the host plant's endogenous auxins than from the fungus auxins. However, seedlings of pine inoculated with mutant strain of *Hebeloma crustuliniforme* that overproduced IAA generated an increased number of EM roots (Gay et al. 1994).

16.4.2   Cytokinins

Cytokinins are N6-substituted aminopurines, including ribosides, ribotides, and glucosides. These are adenine derivatives characterized by their ability to induce cell division in tissue culture in the presence of auxins. The most common cytokinin in plants is zeatin, which is converted to other cytokinins. Over 40 cytokinins have been characterized in plant tissues (McGaw and Burch 1995).

Cytokinins are responsible for the translocation of carbohydrates to the mycorrhizal roots. They also act indirectly on the activity of auxins (Gogala 1991).

Several mycorrhizal fungi have been shown to be capable of producing cytokinins in vitro. However, it is unclear whether these fungi that are capable of producing cytokinins also do so in association with the macrosymbiont (Arshad and Frankerberger 1998). No direct, unequivocal evidence indicates that cytokinins are a prerequisite for the formation of mycorrhizae. However, higher cytokinin levels in mycorrhizal plants have been reported, but the source of increased cytokinin levels in mycorrhizal plants is somewhat unresolved.

Allen et al. (1980) reported higher cytokinin activity in mycorrhizal plants compared with noninfected (control) plants. Similarly, Thiagarajan and Ahmad (1994) reported significantly greater cytokinin content (156%) in mycorrhizal roots compared to nonmycorrhizal roots.

Several other studies confirmed these findings and provided evidence that inoculation with mycorrhizal fungi results in increasing the endogenous cytokinin contents of host plants (Dixon 1989; Danneberg et al. 1992).

In the plant root zone (rhizosphere), there are also plant growth regulators elaborated by microorganisms accompanying mycorrhizae (Strzelczyk and Pokojska-Burdziej 1984) and those originating from the root exudates (Gogala 1991).

Zeatin
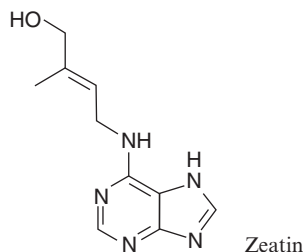
Little is known about the direct effects of these compounds on mycorrhizal fungi. However, Pokojska et al. (1993) showed differences in the effects of plant regulators on mycorrhizal fungi (*H. crustuliniforme*, *Laccaria laccata*, *Rhizopogon vinicolor*) depending upon the kind of hormones, its concentration, and the kind of fungus.

Kinetin inhibited biomass production by *L. laccata* in a liquid medium but it did not inhibit the linear growth of this fungus on agar medium. Reverse results were observed with *R. vinicolor*.

Auxins did not affect the growth of *L. laccata*, but some of them exhibited both inhibitory and stimulatory effects on the growth of *H. crustuliniforme* and *R. vinicolor* depending upon the concentration and type of the medium.

Gogala and Pohleven (1976) have shown that cytokinins promoted the mycelial growth of *S. variegatus* and affected the content of K, Ca, P, and Na in the mycelium of this fungus. In the presence of kinetin, the uptake of Cd, Zn, P by some EM fungi increased significantly (Stegnar et al. 1978).

The importance of auxins and cytokinins in plant growth and development is known. The role of these substances in microorganisms is not elucidated as yet. According to the data obtained from the literature, it can be assumed that auxins and cytokinins do not play the role of hormonal factor in microorganisms (Gogala and Pohleven 1976; Pohleven and Gogala 1986; Gogala 1989; Pokojska et al. 1993).

16.4.3   Gibberellins (GAs)

GAs are tetracyclic diterpenoid acids with an ent-gibberellane ring system. Mevalonic acid is the primary precursor of GAs biosynthesis in plants.

Although the most widely recognized gibberellin is GA3 (gibberellic acid) which is a fungal product, the most active GA in plants is GA1 which is primarily responsible for stem elongation (Arshad and Frankerberger 1998).

Gibberelic acid
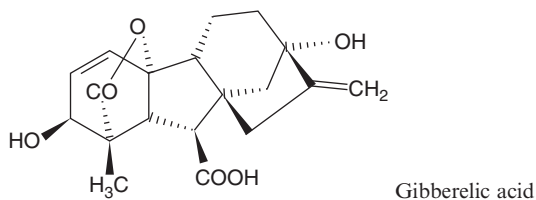
Very little work has been conducted on the detection of GAs released by mycorrhizal fungi. Gogala (1971) detected gibberellin-like substances in culture medium of the EM fungus *Boletus edulis* and Ho (1987) in culture of *P. tinctorius*. Strzelczyk et al. (1975) found gibberellin-like substances produced by *Suillus bovinus*, *H. crustuliniforme*, and *C. graniforme*.

16.4.4 Ethylene

Ethylene is synthesized from methionine in many plant tissues, mostly in response to stress (Arshad and Frankerberger 1998). It is the only hydrocarbon (C_2H_4) with a pronounced effect on plants and is involved in developmental processes, from germination of seeds to senescence of various organs.

$$H_2C{=}CH_2$$
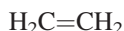
Graham and Linderman (1980) found that EM fungi produced ethylene when grown in medium containing methionine. DeVries et al. (1987) noted an apparent correlation between C_2H_4 production and morphological effects, such as stimulation of lateral root formation by mycorrhizal fungi.

16.4.5 Abscisic Acid

ABA is a sesquiterpene, derived from mevalonic acid. ABA appears to act as much as a promotor (e.g., storage protein), as an inhibitor, and a more open attitude toward its overall role in plant development is warranted (Davies 1995).


Abscisic acid (ABA)
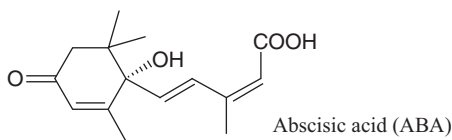
Production of ABA by mycorrhizal fungi has not been demonstrated as yet; however, few studies have investigated the alteration in ABA levels in mycorrhizal-infected plants.

16.5 Sterols

Sterols play an essential role in the physiology of eukaryotic organisms. Sterols are also known as steroid alcohols. They are a subgroup of steroids with a hydroxyl group. They are amphipathic lipids synthesized from acetyl-coenzymes.

Ergosterol is a component of fungal cell membranes, serving the same function that cholesterol serves in animal cells. Ergosterol is used as an indicator of fungal biomass in soil.
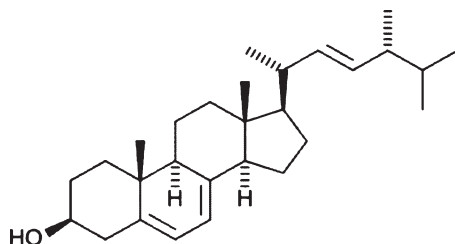
Ergosterol

The composition of fatty acids and sterols in soil lipid fraction is often used as an indicator for the changes of soil microorganisms.

Laczko et al. (2004) performed an experiment in which seedlings of *Pinus sylvestris* and EM fungus *P. tinctorius* were grown separately or combined to form ectomycorrhiza. Fatty acids of the neutral lipid fraction (NLFAs) and the phospholipids fraction (PLFAs) as well as sterol were identified. When grown separately, the two organisms differed strongly with respect to the sterol composition. Sterols had a much higher relative abundance in the fungus in comparison with the plant and the two main fungal sterols, ergosterol and 24-ethyllanosta-8,24(24)-diene-3 beta, 22 zeta-chiol (Et lano 8.24) as well as six minor fungal sterols were not found in the plant roots. When the fungus and plant were brought together, there was a drastic change in the lipid composition of the root.

It was detected that in symbiosis, the fungus transports plant lipids from the symbiotic interface to the extramatrical mycelium. Concerning sterols, the extramatrical mycelium acquired only a small amount of plant-specific sterols. However, its ergosterol content steadily decreased whereas the content of Et lano 8,24 remained high, causing the ratio of these two sterols to decrease from 1:70 to 1:20, whereas in the EM roots, the opposite phenomenon occurred, so that the ratio increased to a value of almost 1:1.

These results showed that an EM fungus may display markedly different lipid composition in its intraradical and extraradical part and highlight a potential role of plant lipid transfer from the root to the fungus in the functioning the of EM symbiosis.

16.6   Conclusions and Future Perspectives

Production of SM is widespread among plants and rhizosphere microorganisms including mycorrhizal fungi. However, conditions in the rhizosphere are often quite variable and many factors, mainly availability of nutrients composition and amount of root exudates as well as interaction between rhizosphere microorganisms, can affect the synthesis of SM by plants and microorganisms associated with plant root.

Research on the role of SM in the initiation development function of mycorrhiza is mainly concerned with two tasks: the determination of their role in the metabolism, growth, and development of the mycorrhizal fungi and the determination of their role in root morphology in the growth of the entire plant and in causing metabolic changes in plants.

Widespread ability of mycorrhizal fungi to produce plant hormones in culture media and induction of mycorrhizal-like changes in response to exogenous application of plant hormones favor the speculation that fungal hormones may have a role in establishment of the symbiotic relation and in the physiology of mycorrhizal plants.

However, physiology of hormones released by the microsymbiont and the role of these metabolites in the symbiotic association are still poorly understood. Very little is known about the molecules regulating the interaction between plants and EM fungi during root colonization.

The role of fungal auxin in ectomycorrhiza has repeatedly been suggested and questioned, suggesting that, if fungal auxin controls some steps of colonized root development, its activity might be tightly controlled in time and in space by plant and/or fungal regulatory mechanisms.

Increase in auxin synthesis or auxin accumulation was noted in most plant–microbe interactions in plant tissues. However, in some interactions (e.g., *P. tinctorius* and *Eucalyptus*) downregulation of the auxin activity in the host plant was observed.

It is assumed that hypaphorine (betaine of tryptophan) might be the specific IAA antagonist.

Despite increasing evidence, the role of flavonoids and other phenolic substances in EM symbiosis is yet contradictory. The change in the balance of plant hormones and other SM have yet to be examined for plant development and first signal for initiation of mutualistic symbiosis. Understanding of these problems could be of great ecological benefit to the agriculture and forestry industry.

A large number of studies have verified that multiplicity of signals and diversity of signaling pathways exist during the establishment of mycorrhizal associations with regulation of symbiosis-specific genes expression.

In presymbiotic phase, plant and their fungal partner secrete signals into soil, mostly SM inducing morphological and physiological changes. The nature of the signals released by the EM symbionts and processes triggering the expression of genes that participate and regulate symbiosis in partner recognition are only the beginning to be understood. Although many genes have been identified in various EM association, the product of which play role in recognition and attachment of the mycobiont on the root surface remains unknown. Many questions concerning the differentiation of plant and fungal symbiotic structure are also poorly recognized. It is interesting to be analyzed how is elicitor's signal achieved depending on the activation factors and which substances participate in this signaling network.

References

Allen MF, Moore TS, Christensen M (1980) Phytohormone changes in *Bouteloua gracilis* infected by vesicular-arbuscular mycorrhizae: I. Cytokinin increases in the host plant. Can J Bot 58: 371–374

Arshad M, Frankerberger WT (1998) Plant growth-regulating substances in the rhizosphere: microbial production and functions. Advan Agron 62:45–151

Asiegbu FO, Johansson M, Woodward S, Hütterman A (1998) Biochemistry of the host–parasite interaction. In: Woodward S, Stenlid J, Karialajnen R, Hütterman A (eds) *Heterobasidion annosum*: Biology, ecology, impact and control. University Press, Cambridge, pp 167–193

Baron C, Zambryski PC (1995) The plant response in pathogenesis, symbiosis, and wounding: variations on a common theme? Ann Rev Genet 29:107–129

Béguiristain T, Lapeyrie F (1997) Host plant stimulates hypaphorine accumulation in *Pisolithus tinctorius* hyphae during ectomycorrhizal infection while excreted fungal hypaphorine controls root hair development. New Phytol 136:525–532

Beyler M, Heyser W (1997) The influence of mycorrhizal colonization on growth in the greenhouse and on catechin, epicatechin and procyanidin in roots of *Fagus sylvatica* L. Mycorhhiza 7:171–177

Brown DE, Rashotte AM, Murphy AS, Normanly J, Tague BW, Peer WA, Taiz L, Muday GK (2001) Flavonoids act as negative regulators of auxin transport in vivo in *Arabidopsis*. Plant Physiol 126:524–535

Danneberg G, Latus C, Zimmer W, Hundeshagen B, Schneider-Poetsch H, Bothe H (1992) Influence of vesicular-arbuscular mycorrhiza on phytohormone balances in maize (*Zea mays* L.). J Plant Physiol 141:33–39

Davies PJ (1995) The plant hormones: their nature, occurence, and functions. In: Davies PJ (ed) Plant hormones: physiology, biochemistry and molecular biology. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 1–12

DeVries HE, Mudge KW, Lardner JP (1987) Ethylene production by several ectomycorrhizal fungi and effects on host root morphology. In: Sylvia EM, Hung LL, Graham JH (eds) Mycorrhizae in the next decade. Institute of Food and Agricultural Sciences, University of Florida, Gainesville, p 245

Ditengou FA, Béguiristain T, Lapeyrie F (2000) Root hair elongation is inhibited by hypaphorine, the indole alkaloid from the ectomycorrhizal fungus *Pisolithus tinctorius*, and restored by IAA. Planta 211:722–728

Dixon RK (1989) Cytokinin activity in *Citrus jambhiri* seedlings colonized by mycorrhizal fungi. Agric Ecosystems Environ 29:103–106

Frankenberger WT, Poth M (1987) Biosynthesis of indole-3-acetic acid by the pine ectomycorrhizal fungus *Pisolithus tinctorius*. Appl Environ Microbiol 53:2908–2913

Garcia-Garrido JM, Ocampo JA (2002) Regulation of the plant defence response in arbuscular mycorrhizal symbiosis. J Exp Bot 53:1377–1386

Gay G (1986) Effect of glucose on indole-3-acetic production by the ectomycorrhizal fungus *Hebeloma hiemale* in pure culture. Physiol Veg 24:185–192

Gay G, Debaud JC (1987) Genetic study on indole-3-acetic acid production by ectomycorrhizal *Hebeloma* species: inter- and intraspecific variability in homo- and dikaryotic mycelia. Appl Microbiol Biotechnol 26:141–146. doi:10.1007/BF00253898

Gay G, Normand L, Marmeisse R, Sotta B, Debaud JC (1994) Auxin overproducer mutants of *Hebeloma cylindrosporum* Romagnési have increased mycorrhizal activity. New Phytol 128:645–657

Gogala N (1971) Growth substances in mycorrhiza of the fungus *Boletus pinicola* Vitt. and the pine-tree *Pinus sylvestris* L. Razprave 14:123–202

Gogala N (1989) Growth substances in root exudate *Pinus sylvestris* – their influence on mycorrhizal fungi (effect of jasmonic acid). Agric Ecosystems Environ 28:151–154

Gogala N (1991) Regulation of mycorrhizal infection by hormonal factors produced by hosts and fungi. Experimentia 47:331–339

Gogala N, Pohleven F (1976) The effect of cytokinins and auxins on the growth of mycorrhizal fungus *Suillus variegatus*. Acta Bot Croat 35:129–134

Graham JH, Linderman RG (1980) Ethylene production by ectomycorrhizal fungi, *Fusarium oxysporum* f. sp. *pini*, and by aseptically synthesized ectomycorrhizae and *Fusarium*-infected Douglas fir roots. Can J Microbiol 26:1340–1347

Higuchi T (1985) Biosynthesis and biodegradation of wood components. Academic Press, Orlando, p 679

Ho I (1987) Comparison of eight *Pisolithus tinctorius* isolates for growth rate, enzyme activity, and phytohormone production. Can J For Res 17:31–35

Horan DP, Chilvers GA, Lapeyrie F (1988) Time sequence of the infection process in eucalypt ectomycorrhizas. New Phytol 109:451–458

Kakiuchi Y, Gàlis I, Tamogami S, Wabiko H (2006) Reduction of polar auxin transport in tobacco by the tumorigenic *Agrobacterium tumefaciens* AK-*6b* gene. Planta 223:237–242

Kampert M, Strzelczyk E (1989) Effect of amino acids on cytokinin-like substances production by micorrhizal fungi of pine (*Pinus sylvestris* L.). Agric Ecosyst Environ 28:219–228

Koide RT, Suomi L, Stevens CM, McCormick L (1998) Interactions between needles of *Pinus resinosa* and ectomycorrhizal fungi. New Phytol 140:539–547

Kottke I, Oberwinkler F (1987) The cellular structure of the Hartig net: coenocytic and transfer cell-like organization. Nordic J Bot 7:85–95

Krupa S, Andersson J, Marx DH (1973) Studies on ectomicorrhizae of pine IV. Volatile organic constituents in micorrhizal and nonmycorrhizal root systems of Pinus echinata Mill. Eur J For Pathol 3:194–200

Laczko E, Boller T, Wiemken V (2004) Lipids in roots of *Pinus sylvestris* seedlings and in mycelia of *Pisolithus tinctorius* during ectomycorrhiza formation: changes in fatty acid and sterol composition. Plant Cell Environ 27:27–40

Lagrange H, Jay-Allemand C, Lapeyrie F (2001) Rutin, the phenolglycoside from *Eucalyptus* root exudates, stimulates *Pisolithus* hyphal growth at picomolar concentrations. New Phytol 150:349–355

Laitinen ML, Julkunen-Tiitto R, Tahvanainen J, Heinonen J, Rousi M (2005) Variation in birch (*Betula pendula*) shoot secondary chemistry due to genotype, environment, and ontogeny. J Chem Ecol 31:697–717

Martin F, Duplessis S, Ditengou F, Lagrange H, Voiblet C, Lapeyrie F (2001) Developmental cross talking in the ectomycorrhizal symbiosis: signals and communication genes. New Phytol 151:145–154

McGaw BA, Burch LR (1995) Cytokinin biosynthesis and metabolism. In: Davies PJ (ed) Plant hormones: physiology, biochemistry and molecular biology. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 98–117

Mekin E, Krupa S (1971) Studies on ectomicorrhizae of pine. II. Growth inhibition of mycorrhizal fungi by volatile organic constituents of *Pinus silvestis* L. (Scots pine) roots. Physiol Plantarum 25:337–340

Molina E, Trappe JM (1982) Patterns of ectomycorrhizal host specificity and potential among pacific northwest conifers and fungi. Forest Sci 28:423–458

Napierała-Filipiak A, Werner A, Mardarowicz M, Gawdzik J (2002) Concentrations of terpenes in micorrhizal roots of Scots pine (*Pinus sylvestris* L.) seedlings grown in vitro. Acta Physiol Plant 24:137–143

Niemi K, Julkunen-Tiitto R, Häggman H, Sarjala T (2007) *Suillus variegatus* causes significant changes in the content of individual polyamines and flavonoids in Scots pine seedlings during mycorrhiza formation in vitro. J Exp Bot 58:391–401. doi:10.1093/jxb/erl209

Peer WA, Bandyopadhyay A, Blakeslee AA, Makam SN, Chen RJ, Masson PH, Murphy AS (2004) Variation in expression and protein localization of the PIN family of auxin efflux facilitator proteins in flavonoid mutatnts with altered auxin transport in *Arabidopsis thaliana*. Plant Cell 16:1898–1911

Pohleven F, Gogala N (1986) The influence of natural cytokinins on the content of K, P, Ca and Na in the mycelium of the mycorrhizal fungus *Suillus variegatus*. Biol Vestn 34:79–88

Pokojska A, Strzelczyk E, Li CY, Rozycki H, Szablewska M (1993) Effect of plant growth regulators on growth of ectomycorrhizal fungi. Cryptogam Bot 4:8–13

Prior C (1976) Resistance by Corsican pine to *Fomes annosus*. Ann Bot 40:261–279

Rupp LA, Mudge KW, Negm FB (1989) Involvement of ethylene in ectomycorrhiza formation and dichotomous branching of roots of mugo pine seedlings. Can J Bot 67:477–482

Schützendübel A, Polle A (2004) Plant responses to abiotic stress: heavy metal-induced oxidative stress and protection by mycorrhization. J Exp Bot 53:1351–1365

Slankis V (1950) Effect of α napthaleneacetic acid on dichotomus branching of isolated roots of *Pinus silvestris*. Physiol Plantarum 3:40

Slankis V (1973) Hormonal relationships in mycorrhizal development. In: Marks GC, Kozlowski TT (eds) Ectomycorrhizae: their ecology and physiology. Academic Press, New York, pp 231–298

Smith WH (1987) The atmosphere and the rhizosphere: linkages with potential significance for forest tree health. In: Blasser RO (ed) Technical Bulletin of national council of the paper industry for air and stream improvements, vol. 527. New York, pp 30–94

Stegnar P, Gogala N, Pohleven F (1978) The uptake of cadmium, zinc, phosphorus, and plant hormone kinetin by ectomycorrhizal fungi. Acta Bot Croat 37:67–73

Strzelczyk E, Pokojska-Burdziej A (1984) Production of auxins and gibberellin-like substances production by mycorrhizal fungi, bacteria and actinomycetes isolated from soil and mycorrhizosphere of pine (*Pinus sylvestris* L.). Plant Soil 81:185–194

Strzelczyk E, Sitek JM, Kowalski S (1975) Production of gibberelin-like substances by fungi isolated from mycorrhizae of pine (*Pinus silvestris* L.). Acta Microbiol Pol 7:145–153

Taylor LP, Grotewold E (2005) Flavoniods as developmental regulators. Curr Opinion Plant Biol 8:317–323

Thiagarajan TR, Ahmad MH (1994) Phosphatase activity and citokinin content in cowpeas (*Vagna unguiculata*) inoculated with a vesicular-arbuscular mycorrhizal fungus. Biol Fertil Soils 17:51–56

Varese TC, Portinaro S, Trotta A, Scannerini S, Luppi-Mosca AM, Martinotti MG (1996) Bacteria associated with *Suillus grevillei* sporocarps and ectendomycorrhizae and their effects on in vitro growth of the mycobiont. Symbiosis-Rehovot 21:113–129

Weiss M, Mikolajewski S, Peipp H, Schmitt U, Schmidt J, Wray V, Strack D (1997) Tissue-specific and developmental-dependent accumulation of phenylpropanoids in larch mycorrhizas. Plant Physiol 114:15–27

Weiss M, Schmidt J, Neumann D, Wray V, Crist R, Strack D (1999) Phenylpropanoids in mycorrhizas of the *Pinaceae*. Planta 208:491–502

Werner A (1993) Pathological anatomy of thin woody roots of Scots pine invaded by *Heterobasidion annosum* (Fr.) Bref. Arboretum Kórnickie 38:113–129

Wink M (2008) Plant secondary metabolism: diversity, function and its evolution. Nat Prod Communicat 3:1205–1216

Zhi-lin Y, Chuan-chao D, Lian-qing C (2007) Regulation and accumulation of secondary metabolites in plant-fungus symbiotic system. Afr J Biotechnol 6:1266–1271