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0014-4754/91/040321-11$1.50 + 0.20/0

Regulation of mycorrhizal infection by hormonal factors produced by hosts and fungi

N. Gogala

Department of Biology, Biotechnical Faculty, University of Ljubljana, Aškerčeva 12, 61001 Ljubljana (Yugoslavia)

Summary. An overview of current research on hormonal factors produced by plants and fungi in mycorrhizal associations is presented. On the one hand, growth hormones in roots and their exudates influence the metabolism and growth of fungi. On the other, fungal hormones influence root morphology, metabolic changes and the growth of the entire plant.

Key words. Mycorrhiza; plant hormones; fungi; root exudate; roots; growth substances.

Introduction

There are few topics in plant physiology and ecology with as many open questions as research on mycorrhizas. The one in focus here concerns the regulation of mycorrhizal infection, and in particular the role of plant hormones. According to Allen[1], the formation of mycorrhizas represents a special adaptation to surviving in unsuitable conditions and under stress in the habitat. In general, adaptations of plants to changes in the external environment involve changes in plant growth and development and are controlled by plant hormones[114]. It is no wonder, therefore, that the role of plant hormones has been a focus of attention of investigators of mycorrhizas since the discovery of the auxins. Later, after the discovery of other growth regulators, their presence in mycorrhizal associations was also investigated. Much research work has already been done, but even more is needed in the next decades as new techniques are developed.

The present review summarizes current investigations on plant hormones in root exudates and their influence on mycorrhizal fungi, and also on the role of fungal hormones in mycorrhizas.

Growth substances in roots and root exudates

Mycorrhizal fungi and other organisms in the rhizosphere show negative or positive growth reactions in response to the levels of nutritive substances, and other factors that affect growth, present in the substrate. These substances are thought to be important for the establishment of mycorrhizal associations. In this connection, root exudates have been much investigated (see review articles by Curl and Truelove[14] and Rambelli[83]). It is well known that root exudation is a phenomenon common to all higher plants.

In contrast to the many reports on the content of carbohydrates, amino acids and other organic acids, and vitamins in root exudates, there are only few papers concerning plant hormones and their influence on the growth and metabolism of mycorrhizal fungi. It should be noted that for the understanding of the metabolism of mycorrhizal roots, data on hormones in root tissues are also important.

Many results on the influence of exudates on the growth of fungi were published in the classic papers of Melin[69]. He concluded from his experiments that the roots of pine, and also of many other plant species, produce not only vitamins and amino acids, but also a substance called 'M-factor', which contains one or more growth-promoting metabolites which are essential to the growth of the fungal symbionts. Melin thought that the M-factor contained at least two substances; one could diffuse through plasma membranes and the other was bound within the cells. M-factor in higher concentrations inhibited the growth of mycelia and this effect was more pronounced after addition of the pine root extract. After publication of these results, interest in the substances contained in root exudates increased, but nobody was able to prove clearly which substance was responsible for the effects of M-factor. Substances suggested by various authors are NAD (Nilsson in Melin[69]) or cytokinins[43-45] or IAA (indole-3-acetic acid), produced by fungi in interaction with living roots[24,25].

Björkman[8,9] found in the epiparasitic plant *Monotropa hypopitys* a substance which promotes the growth of symbiotic fungi and other mycorrhizal fungi. He named it 'tuburcin', compared it to the Melin's[69] M-factor, and proposed that it had the role of an attractant for the mycorrhizal fungi. Gogala[44,45] compared the effects of cytokinins from a root exudate of *Pinus sylvestris* and from roots of *Monotropa hypopitys* on the growth of *Boletus edulis*, and concluded that they are important parts of the complex influences of pine growth substances on the mycorrhizal fungi. Cytokinin-like substances were identified by paper or thin layer chromatography and bioassay techniques.

Higher intensities of light and a long photoperiod promote the exudation of cytokinins into the rhizosphere[46]. High levels of light intensity and temperature also increase the exudation of other substances[112]. According to Bowen[10], exudation of substances from the roots is directly related to the nutritional state of the plants. The exudation is greater in plants grown in a solution lacking nitrogen and phosphorus.

Cytokinins were determined in exudates of tomato plants[15], in their roots[18], in corn[23] and pea roots[94], in pressure exudates of soybean roots[57], root exudates of *Xanthium strumarium*[58], and in the Douglas fir xylem extrudate[19], to list only some.

M. Kovač (personal communication) detected a cytokinin-like activity in exudates from *Pinus sylvestris* seedlings. Samples were purified by reversed phase HPLC and analyzed by an *Amaranthus* bioassay. The peak with the highest biological activity was identified as zeatine riboside by gas chromatography and subsequent mass spectrometry (GC-MS). Low biological activity was also detected in the fraction which co-chromatographed with isopentenyladenosine, but further confirmation of this is needed. Much higher cytokinin-like activity was detected in exudates of seedlings grown at 18-22 °C than in those grown at 6 °C. In sour orange, the cytokinin production of mycorrhizal plants was more than twice that of non-mycorrhizal ones[20].

Vančura[113] reported that both the quantity and the qualitative composition of the root exudates change during plant ontogeny. These observations should be confirmed for the exudation of plant hormones as well.

Auxins in root exudates and in root tissue have been rather thoroughly investigated, especially because of their possible role in gravitropism[22]. Abscisic acid, also present in roots, does not influence the growth of mycorrhizal fungi. Jasmonates, on the other hand, which are also present in root tips, inhibit the growth of mycorrhizal fungi, and deserve further attention[47,48]. Jasmonic acid (JA) and its methyl-ester have been the subject of many recent physiological investigations because it has been discovered that they can act as growth regulators. They inhibit growth and promote aging of green plants. JA is a strong inhibitor of the growth of mycorrhizal fungi even at low concentrations (10 µg/ l). Nevertheless, it is only a partial inhibitor in the presence of growth stimulators[48]. Miersch (personal communication) studied the concentration of JA by radioimmunoassay in *Pinus sylvestris* roots. He detected JA only in non-mycorrhizal roots of 10-day-old seedlings and in long roots of adult trees, but he found no JA in mycorrhizal roots.

Župančič and Gogala[115] reported that natural auxins and gibberellins in pine tree seedling exudates inhibit the growth of *Suillus variegatus*.

Influence of plant hormones and secondary substances on the growth and metabolism of mycorrhizal fungi

Among the plant growth regulators, cytokinins are probably the most important for mycorrhizas because of their influence on the growth and development not only of the higher plants themselves, but also of the mycorrhizal fungi. As investigated by Crafts and Miller[13], the content of naturally occurring cytokinins present in some of these fungi was very low, while some other species were not able to produce them at all. Therefore it is interesting that cytokinins, present in large amounts in the pine tree root exudate, promote mycelial growth of mycorrhizal fungi[49]. The fresh weight of treated mycelium is significantly higher than in the control mycelium, but differences in dry weight are less pronounced. The effect of cytokinins is not linearly related to their concentration and in supraoptimal concentrations they even inhibit mycelial growth. Cytokinins also have an important function in diluted media: they compensate for the negative effect of the dilution on the growth of a pure culture of the mycelium[46].

Cytokinins influenced the content of K, Ca, P and Na in the mycelium of the fungus *Suillus variegatus*[79]. Kinetin increased the uptake of Cd, Zn, Ca and P significantly in some mycorrhizal fungi[97, 100].

Pohleven[77, 78] has studied the influence of cytokinins on K, Ca, P and Na transport in the mycelium of *Suillus variegatus*. Kinetin, zeatin, zeatin riboside, and 2-isopentenyl adenine had specific effects on each ion. In general, at the beginning of mycelial growth in vitro, cytokinins increased the uptake of ions by the mycelium and later also their transport along hyphae and back into the substrate. With the method of electron paramagnetic resonance (EPR) Pohleven found that cytokinins increase the fluidity of hyphal membranes. This change in microviscosity could influence ion and water transport processes through the fungal membrane. It is still unclear how cytokinins influence ion absorption. Current research, done so far not with fungi but with higher plants, shows several different possible pathways of action: through protein and/or enzyme synthesis, through membrane permeability, or through the electrogenic proton pump with all its attendant carriers of individual ions.

In comparison to these results, the study of Pohleven and Križaj is interesting (unpublished observations). They stimulated mycelial growth of *Suillus variegatus* with the root exudate from *Pinus sylvestris* seedlings or, in separate, parallel experiments, with a continuous direct electrical current (14-day treatment). The strength of the current was 1.5 microamperes, which was chosen to mimick that of the extracellular loop of the endogenous electrical currents normally found around living root cells. The optimal concentration of the root exudate and the electrical current had a remarkably similar effect on most of the parameters examined (mycelial mass and potassium, sodium and calcium uptake). The rate of growth and the amount of accumulated K and Ca was increased by 20–30%. An additional preliminary observation from this study is that the concentration of inorganic phosphates in an electrically or chemically stimulated mycelium falls drastically, to the level of about 30% of the control values, a change which might reflect the higher metabolic activity of the fungus. Pohleven and Križaj interpret these results as indications of the influence of cytokinins or electric currents on the membrane transport mechanisms.

Interestingly, the rate of polar auxin transport increases with electrical stimulation in the microampere range[84] and, furthermore, the inhibitors of auxin transport inhibit the current-induced effects on cellular growth and differentiation[85]. Auxins are also thought to be effectors of the proton pump[54] and they tend to increase the K/Na selectivity of transport[111]. The complexity of the interaction is even more evident if we consider the existence of fungal endogenous currents[50] and the fact that plant roots themselves tend to be influenced by currents in the microampere range[67].

Data on the influence of environmental factors such as light, temperature, ions in substrates, etc. on the exudation of cytokinins and other hormones suggest a possibility of combining two theories of the regulation of mycorrhizae: the carbohydrate theory of Björkman[9] and the hormonal theory of Slankis[96]. The same factors which promote the exudation of carbohydrates and other assimilates also stimulate the exudation of hormones. These factors enhance the supply of energy, vitamins and precursors of other active substances for the heterotrophic symbiont, and simultaneously influence the uptake and transport of water and minerals by mycelia. The idea of a mutual complementation of the two mechanisms is supported by the results of Rudawska[88]. She supplemented a synthetic medium for cultures of mycorrhizal fungi with powdered non-mycorrhizal roots from *Pinus sylvestris*, grown either under normal or under reduced daylight. Fungal cultures supplemented particularly with root-powder from plants grown in full daylight showed a growth increase and enhanced levels of auxin and cytokinin synthesis. It was also shown that the activity of fungal IAA-oxidase was inhibited by aqueous root extracts, by root exudates, and by filtrates from fungal cultures supplemented with the root-powder derived from pine seedlings grown in full daylight. It is suggested, therefore, that some factor in pine roots, possibly one of the polyphenols many of which can strongly influence auxin activity and metabolism[93] accumulates under strong light conditions. This factor favors mycelial growth and the production of auxins and cytokinins in cultures of mycorrhizal fungi.

A special field in mycorrhizal research is the search for factors stimulating spore germination. Fries[29–31] reported that in the mycorrhiza-formers, chiefly agarics and boleti, germination can often be induced by exudates from tree roots or certain yeasts, and in species of *Lec-*

cinum by the exudate formed by the mycelium itself. Reviews[29, 56] of the abundant literature in this field lead to the conclusion that there is a substantial diversity of conditions for spore germination in individual species of fungi. Unfortunately, there are only a few identified substances in roots or root exudates which can trigger germination. Several diterpene resin acids possess the capacity to induce spore germination in ectomycorrhizal species of the genus *Suillus*[32, 33]. Abietic and palustric acids are the most active; dehydroabietic, levopimaric, isopimaric and neoabietic acids are less active, and pimaric and pinifolic acid proved to be inactive. Because of the different influences of pine root exudates and extracts on various fungal species. Fries[32] concludes that these extracts contain not only diterpene resin acids but also other active compounds.

To our knowledge, there are no data about the influence of plant growth substances on spore germination of mycorrhizal fungi.

Plant-growth substances produced by mycorrhizal fungi

Knowledge of the presence of plant-growth substances in fungi has increased rapidly with the development of detection and identification methods, but the question of their influence on roots and mycorrhizal plants is still a matter of controversy. Investigations up to 1972 are covered in a review by Slankis[96], who contributed much to our understanding of the influence of auxins on root morphology in mycorrhizal plants and proposed a hormonal theory of the regulation of mycorrhiza formation. Nylund[76] noted in a critical comparison of Slankis' hormone theory[96] with Björkman's carbohydrate theory[9] the following lines of evidence for the hormonal theory:

- Root structures resembling ectomycorrhizas can be induced in pine roots either by fungal exudates or by supraoptimal concentrations of synthetic auxins.
- Terminating the periodic supply of auxins or exudates results in renewed elongation of the roots and termination of the mycorrhiza-like status, followed by the formation of root hairs.
- Ectomycorrhizas undergo the same type of morphogenesis when subjected to high nitrogen concentrations, which arrest the formation of new mycorrhizas and cause termination of the symbiosis.
- IAA and other indole compounds abound in mycorrhizal roots, while only traces are present in nonmycorrhizal ones.
- Production of IAA in vitro by the mycorrhizal fungus *Suillus bovinus* was suppressed in a medium rich in nitrogen.
- Carbohydrate concentrations in the root did not vary with light exposure or with the fertility of the medium, as Björkman[9] had claimed. Instead, differences in carbohydrate concentration could be attributed to the effects of the established symbiosis.

Below, the evidence for plant hormone production by mycorrhizal fungi is discussed.

Auxins

Slankis[96] developed his theory on the role of auxins in the development and ramification of mycorrhizal short roots on the basis of experiments with synthetic auxins or with crude fungal exudates. The presence of auxins in mycorrhizal fungi was first detected by bioassay techniques[10, 72]. These methods are still important because they offer direct evidence for auxin-type physiological activities. Of course, auxins are nowadays quantitatively and qualitatively determined by combinations of modern analytical methods including gas chromatography (GC), high performance liquid chromatography (HPLC) and mass spectrometry, as well as immunological methods (RIA, ELISA).

Ulrich[110] reported that out of 11 fungi only *Suillus variegatus* and *S. granulatus* produced indole-3-acetic acid (IAA) without tryptophan being present in the culture medium. Most ectomycorrhizal fungi release IAA only to media containing tryptophan[21, 37, 38, 72, 109] or tryptophan precursors[39, 61]. Gay et al.[39] reported that *Hebeloma hiemale* synthesized IAA when incubated in vivo with 0.1 mM tryptophan. The amount of IAA synthesized per mg of fungal protein increased as a function of tryptophan concentration in the incubation solution but it was only 4 times higher when the tryptophan concentration was increased tenfold. The IAA-synthesizing enzyme activity in vitro increased even less, only by a factor of 2, as a function of the tenfold increase of tryptophan. These results indicate that precursor availability in root exudates is probably one of the main limiting factors for IAA synthesis by ectomycorrhizal fungi under symbiotic conditions. They also demonstrate that precursors other than tryptophan (tyrosine, phenylalanine, shikimic acid) can be metabolized to IAA.

The study of Pokojska and Strzelczyk[80] shows that organic acids affect the production of auxin-like substances (determined with bioassay and GC) by mycorrhizal fungi growing in L-tryptophan-supplemented media (0.2 g/l). Fumaric acid decreased the production of these substances in *Rhizopogon luteolus*. Pyruvic acid and 2-oxoglutaric acid inhibited their production completely in the same fungus and also in *Amanita muscaria*. On the other hand, pyruvic acid markedly enhanced the production of auxin-like substances in *Suillus bovinus*. The authors suggest that the influence of other organic acids should be considered in future studies.

Rudawska[89] has shown that some amino acids (glutamine, asparagine, arginine, glycine, alanine and proline) diminished the content of auxins in fungal filtrates in the presence of tryptophan in the medium.

During mycelial cultivation on the basal liquid nutrients it was noticed[109] that among 15 species belonging to the Boletaceae (*Suillus, Boletus, Xerocomus*) the auxin production correlated positively with the output of dark pigments exuded into the culture medium (quinones and/or polyphenols). Tomaszewski et al.[109] argued that polyphenols are known to inhibit the IAA peroxidase activity, and it appears that they might control the auxin level. Auxin accumulation may depend on polyphenols or quinones which may act as protectors against its destruction. If this is true, it could be of interest in developing non-destructive methods of auxin extraction and identification. It is possible that more auxin is usually detected in fungi which exude more polyphenols. These results should be checked with HPLC and ELISA methods. Strzelczyk and Pokojska[102] found that most often vitamins retarded auxin production. Pyridoxine was the most inhibitory. Only *Suillus bovinus* in the presence of biotin and pantothenic acid, and *Amanita muscaria* grown with thiamine and biotin, produced more auxins. Ek et al.[21] studied the production of IAA with GC and MS in 16 mycorrhiza-forming fungi. Tryptophan was added to the MNM medium. Results indicated large differences in the ability of the fungal strains to produce IAA. *Pisolithus tinctorius*, strain 185, produced the largest amounts of IAA (19 mg IAA gd.wt^{-1} of growth medium and 0.8 mg IAA gd.wt^{-1} mycelium).

The biosynthesis and exudation of IAA by *Pisolithus tinctorius*, strain 471, was studied in the presence of tryptophan in the medium by HPLC, GC – MS and ELISA[26] and was found to be 4 – 5 µmoles/l.

Ho[60] recently demonstrated that eight isolates of *Pisolithus tinctorius* produce IAA on a mineral medium even without tryptophan. The strain 359 was the slowest-growing in culture and produced significantly more indoleacetic acid (approx. 1 mmol gd.wt^{-1}) than all other isolates. Six isolates of *Laccaria laccata* also differed significantly in the production of IAA[59].

The biosynthesis of IAA was studied in the fungus *Hebeloma hiemale*[35, 39, 87], and also in other *Hebeloma* species[37]. IAA, indolyl-5-carboxylic acid and indole-3-aldehyde were detected by mass spectrometry in 7-week-old, tryptophan-containing culture filtrates[87]. *Hebeloma hiemale* did not release detectable amounts of IAA when cultivated on a medium containing no precursors or supplemented with 1 mM phenylalanine, 1 mM tyrosine or 1 mM shikimic acid. Accumulation of IAA was also low when the supplement was 1 mM of anthranilic acid[39].

Gay and Debaud[37] studied interstrain variability between 11 wild strains of *Hebeloma cylindrosporum,* and also investigated intrastrain variability using 20 homokaryotic and 50 controlled dikaryotic mycelia belonging to the progeny of one laboratory fruiting strain of this species. In the interstrain study, no correlation was detected between the IAA – synthesizing activity of mycelia and their taxonomic position, their geographic origin, or their host plants. The results of the intrastrain study demonstrate that variability of IAA-synthesis by *H. cylindrosporum* has a genetic basis.

Gay[34] also studied the effect of glucose on IAA production by *Hebeloma hiemale* in pure culture. An increase of its concentration in the nutrient solution from 0.55 mM to 30 mM enhanced mycelial growth and decreased IAA accumulation in culture filtrates. He provided evidence that the inhibition of IAA accumulation in the culture filtrates by high glucose concentrations is due in a large part to a reduced production of enzymes.

The amount of auxines synthesized depends also on the concentration of ions in the medium. Rudawska[90] found that *Suillus bovinus, S. luteus* and *Rhizopogon luteolus* produce more auxin in media without phosphorus. Very low levels of auxins were observed in the presence of nitrate ions in the media of the tested fungi.

In connection with all of these results, we should not neglect research on the ability of other microorganisms living in the rhizosphere to synthesize auxins. They can significantly influence the metabolism and distribution of hormones (see Garbaye, this issue). Kampert et al.[66] investigated the production of auxins by coryneform bacteria, isolated from the roots of *Pinus sylvestris* seedlings. Almost all isolates were capable of producing auxins in tryptophan-containing media, as could be detected by bioassay. Frankenberger and Poth[27] developed a method for the separation of substituted indole derivatives by use of ion suppression reversed phase HPLC in a culture of a soil bacterium (pseudomonad). IAA is considered to be one of the major auxins produced by soil microflora. A number of bacteria (pleomorphic types identical or similar to *Arthrobacter globiformis*, and spore-forming organisms, mainly *Bacillus circulans*) were studied by Strzelczyk et al.[104]. All bacteria and actinomycetes produced auxins in tryptophan-containing media.

The synthesis of auxin-like substances by the microflora in the rhizosphere of pine seedlings was found to be higher in the presence of sodium succinate than in the presence of other carbon sources[81].

Indole-3-ethanol and indole-3-acetic acid were identified by GC and MS in a mycelium isolated from infected roots of *Ophrys lutea* and in the liquid medium[5]. Investigations of auxins in the ericoid mycorrhizal fungus *Pezizella ericae* suggest its IAA synthesizing ability in the presence of precursors in the medium[7].

Non-mycorrhizal fungi associated with the roots of forest trees (*Mycelium radicis atrovirens* and *Trichoderma viride*) also produce auxins and other growth substances in the presence of tryptophan, and only trace amounts of them in a tryptophan-free medium[107].

Cytokinins

Miller[70] isolated and crystallized zeatin and zeatin riboside from media in which *Rhizopogon roseolus* had been cultured. Another presumed ribonucleotide was also detected. Crafts and Miller[13] detected production of cytokinins by several mycorrhizal fungi, among them *Rhizopogon ochraceorubens* and *Suillus punctipes*, and they identified them as trans-zeatin and trans-ribosylzeatin. Miura and Hall[71] have demonstrated that N^6-(Δ^2-isopentenyl) adenosine is a key compound in the biosynthesis of trans-ribosyl-zeatin in *Rhizopogon roseolus. R. roseolus* secretes the t-RNA component into the culture medium[68]. Gogala[44] reported that fruiting bodies, mycelia and also culture media of *Boletus edulis* var. *pinicolus* contain substances with cytokinin activity. Rudawska[88] found variable amounts of cytokinin production in cultures of mycorrhizal fungi.

According to Ho[60], eight isolates of *Pisolithus tinctorius* produced cytokinins and the strain 359, which was most efficient in production of IAA, also produced significantly more cytokinins. The same author[59] detected cytokinins in six isolates of *Laccaria laccata*. Kampert and Strzelczyk[62] found that almost all fungi isolated from mycorrhizas of *Pinus sylvestris* also produced cytokinins. The presence of ammonium in the medium causes a decrease in cytokinin production by mycorrhizal fungi[90]. Phosphorus has no effect on cytokinin production. Amino acids and urea diminish the content of cytokinins (as well as auxins) in the fungal filtrate[89]. However, lysine stimulates the formation of cytokinin-like substances by *Rhizopogon luteolus*[65]. In *Suillus bovinus* only alanine, out of a number of amino acids used, affected the production of cytokinins[65].

Some vitamins of the B-group influence the production of cytokinins in *Rhizopogon luteolus* and *Suillus bovinus*[101]. Pyridoxine stimulated the production by *R. luteolus; S. bovinus,* however, produced these substances only if thiamine and biotin were present.

Other microorganisms, including some bacteria and Actinomycetes living in the mycorrhizosphere, produce cytokinins or influence cytokinin production by mycorrhizal fungi[63, 64, 74, 75, 104, 106, 107].

Gibberellins

Little is known about gibberellins in mycorrhizal fungi. Gibberellin-like substances were detected in fungi isolated from a mycorrhiza of *Pinus sylvestris*[108]. Gogala[42, 44] detected gibberellins in *Boletus edulis* var. *pinicolus* fruitbodies and in mycelia. Several strains of *Pisolithus tinctorius* produced gibberellins[55, 60]. Gibberellins were also detected in filtrates of *Telephora terrestris*[55]. Bacteria and Actinomycetes in the mycorrhizosphere produce gibberellins as well[81, 103]. Strzelczyk et al.[112] detected gibberellins in non-mycorrhizal fungi associated with the roots of forest trees.

The levels of gibberellins were determined by bioassay methods in all the studies cited here.

Ethylene

Ethylene production by ectomycorrhizal fungi has been investigated only recently. Graham and Linderman[51] conducted comparative studies on ectomycorrhizal fungi and *Fusarium oxysporum* to determine the ability of these fungi to produce ethylene in vitro and in aseptic synthesis cultures with the Douglas fir. *Cenococcum geophilum, Hebeloma crustuliniforme* and *Laccaria laccata* produced ethylene in vitro in MN-liquid media only if 2.5 – 10 mM methionine were added. *Pisolithus tinctorius* failed to produce ethylene unless the cultures had fresh methionine added before ethylene assay. An additional 19 ectomycorrhizal fungi plus five isolates of *Fusarium oxysporum* f.sp. *pini* all produced ethylene either in media containing methionine or in renewed media. Ethylene was produced by aseptically grown Douglas fir seedlings inoculated with *C. geophilum, H. crustuliniforme* and *L. laccata.*

Fusarium-inoculated seedlings produced more ethylene, sooner, than those inoculated with mycorrhizal fungi. DeVries et al.[16] reported an intensive production of ethylene in Scots pine seedlings infected with *Laccaria laccata.*

Strzelczyk et al.[107] detected ethylene production by the non-mycorrhizal fungi *Mycelium radicis attrovirens* and *Trichoderma viride*, both of which produced it when methionine was added to the medium.

The influence of fungal growth substances on the development and function of mycorrhizae

A review of data on hormones in mycorrhizal fungi shows that auxins have been studied far more than the others. This is certainly due to Slankis' hormonal theory of mycorrhizal development[96]. The starting point of many investigations was his assumption of the influence of auxins on root morphology and their influence on carbohydrate transport. Unfortunately, there are not many data on the physiological activity of fungal hormones during the growth and development of symbiotic partners. Such investigations are especially difficult in vesicular-arbuscular mycorrhizas (VAM), where the fungus cannot be studied separately and analyses are restricted to comparisons between mycorrhizal and non-mycorrhizal plants. It is therefore understandable that Hacskaylo[53] stated "I have heard it said that what we know about plant hormones in mycorrhizal relationships could be put on the back of a postage stamp".

In evaluating the role of fungal hormones in mycorrhizas the results of the influence of synthetic hormones and extracted natural substances upon the growth, development and metabolism of higher plants are of great help. The theory of Slankis about the role of auxins in the formation of mycorrhizal associations is supported by the results of some authors[10, 11, 44], but he was also criticized by others[56, 86]. Gay[36] reports that *Hebeloma hiemale* can induce rhizogenic effects in *Pinus halepensis* even at very low tryptophan concentrations, which hardly allow any detectable IAA production in an isolated culture. Therefore, he questions, in line with Ritter[86], that mycorrhizal fungi can produce physiologically relevant amounts of IAA under natural conditions. However, the growth of mycelium on a rich medium promotes growth and primary metabolism, but not synthesis of secondary substances such as IAA. Therefore, the synthesis of IAA might be more important for the fungus when the nutrient conditions are suboptimal. This corresponds to Slankis' claim[96] that the absence of mycorrhizas in rich soil can be explained by inhibition of auxin synthesis in fungi under such conditions. Indeed, Rudawska[90] reported auxin synthesis to be higher in a medium deficient in phosphorus and nitrogen. Fungi can also act on the enzymes in the roots responsible for the decay of IAA, and can thereby influence its concentration[56].

It is of interest to consider whether the influence of IAA on root morphogenesis is connected with the production of ethylene. Auxin stimulates ethylene production by pine root cultures[73, 91]. The developmental response of roots to mycorrhizal inoculation might be at least partially due to plant ethylene production induced by fungal auxins. In fact, we must consider the hypothesis that auxins influence root system development indirectly through their effect on root ethylene biosynthesis[73].

Hormone production by mycorrhizal fungi has been implicated in the improved rooting of cuttings. Ectomycorrhizal fungi stimulate the rooting of various cuttings[11, 12, 46]. Application of the synthetic auxins, indole butyric acid (IBA) and naphthalene acetic acid (NAA), stimulate lateral root formation of container-grown Douglas firs[95] and black oak seedlings[6].

Substantial differences have been found in the hormone-synthesizing ability of various fungal species, and also between single strains within one species[59, 60]. The variations in the production of hormones may be related to the variability in ectomycorrhizal development[60].

Plant physiologists have shown that sites of auxin and cytokinin synthesis can act as attraction centers for inflow of assimilates[114]. The mycorrhizal root as a whole, with its high content of such substances, could be similarly considered as such an attraction center. Hormones could also act on the formation of cell walls in the host roots, and the change of the cell wall structure could enable penetration of the Hartig net hyphae. IAA from fungi may also produce a sensitization of the root tissue and a higher susceptibility to mycorrhizal infection, and may even influence the expression of fungal genes[36].

Some effects of VAM on the hosts could also be hormonal. VAM formation can increase the level of cytokinin in the host plant and change the level of abscisic acid and gibberellin-like substances[2, 3, 17, 20, 41, 98]. Application of exogenous IAA increased arbuscular development in roots of the cowpea[52]. *Glomus mosseae* has been shown to synthesize phytohormones[4].

Increased hormone levels in mycorrhizal plants would certainly have a significant impact on plant growth and development and could substantially affect the host's growth response to mycorrhizal infection. However, there is as yet insufficient evidence to determine whether VAM fungi can indeed produce hormones; or if they do, whether there is a direct transfer of fungal hormones to the host, or whether the symbiotic association stimulates host hormone production[82].

In addition to all these open questions, there is a very important weak link: we do not know much about the behavior of these growth substances under in vivo conditions, since a huge majority of the experiments were done in vitro. All microorganisms present in the rhizosphere contribute with their metabolism to interactions with other organisms and all the substances produced by them. A series of investigations give evidence for such interactions[28, 64, 66, 74, 75, 81, 103–107].

Last but not least, we must find new methods for further research to show whether fungal growth substances act directly upon mycorrhizal partners, and whether there are direct connections between the metabolic activities of the two partners. According to Gay[36], one possibility for achieving this aim is to use mutants that are unable to synthesize hormones or else produce abnormal quantities of hormones. Mudge[73] suggests the use of selective inhibitors or yet other approaches to distinguish between phytohormone production by hosts and symbionts in the mycorrhizal systems, to be applied only to either of the organisms alone. Genetic engineering applied to mycorrhizal fungi also might offer modes of research leading to the elucidation of interactions between plant hosts and fungal symbionts[40].

Conclusions

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

Present results indicate that an overlap exists between the hormonal theory[96] of mycorrhiza regulation and both the carbohydrate[9] and the mineral theories[99] (fig. 1). Hormones influence the transport of water and ions,

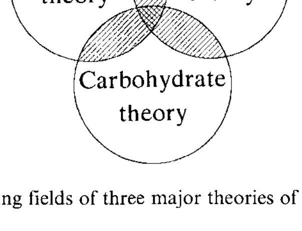

Figure 1. Overlapping fields of three major theories of mycorrhiza regulation.

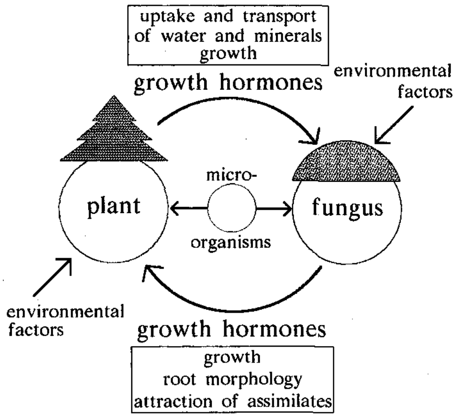

Figure 2. Hormonal influences between mycorrhizal symbionts in natural ecosystems.

carbohydrates and other assimilates. But hormonal levels are influenced in turn by these factors as well as by other organisms in soil with their own metabolic activity, and also by environmental factors such as light and temperature (fig. 2).

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0014-4754/91/040331-10$1.50 + 0.20/0

Assimilation of mineral nitrogen and ion balance in the two partners of ectomycorrhizal symbiosis: Data and hypothesis

C. Plassard, P. Scheromm[a], D. Mousain and L. Salsac

Laboratoire de Recherches sur les Symbiotes des Racines, INRA-ENSA, 1 place Viala, F-34060 Montpellier Cedex 1 (France), and [a]Laboratoire de Technologie des Céréales, INRA, 2 place Viala, F-34060 Montpellier Cedex 1 (France)

Summary. Assimilation pathways of mineral nitrogen and ion balances of the two partners of ectomycorrhizal symbiosis (fungi and woody plants) are reviewed. Data are presented about the partners both in pure culture and in mycorrhizal association. The two forms of mineral nitrogen, ammonium and nitrate, differ in their mobility in the soil, their transport into the cells, their uptake rates by plants and their assimilation pathways. These metabolic differences are related to differences in adjustment of ion balances and carbon metabolism under conditions of nitrate or ammonium nutrition. The data obtained on the partners of ectomycorrhizal symbiosis are discussed from this point of view and the observations composed with those on herbaceous angiosperms.

Key words. Nitrate; ammonium; nitrate reductase; ion balance; organic acids; carbon metabolism; ectomycorrhizal association; woody plants; ectomycorrhizal fungi.

Ammonium and nitrate are the major mineral nitrogen sources used by plants. The uptake and the assimilation of these nutrients cause modifications of the ion balance of tissues, with the production of H^+ and OH^- ions. Mechanisms for the metabolic adjustment of ion balance in herbaceous plants have been reviewed elsewhere[71,87]. In this article, the recent findings about the capacities for and the consequences of the use of these two mineral nitrogen sources by ectomycorrhizal fungi and forest trees are presented and discussed.