SIGNALLING IN ARBUSCULAR MYCORRHIZA:
FACTS AND HYPOTHESES

Horst Vierheilig and Yves Piché

1. INTRODUCTION

The arbuscular mycorrhizal symbiosis is an association between plant roots and fungi. Arbuscular mycorrhizal fungi (AMF) colonize roots improving plant nutrition mainly by transferring phosphate (P) from the soil to the plant, whereas plants provide the fungi with carbohydrates (Smith and Read, 1997). In contrast to the rhizobial symbiosis with a host range limited to the Leguminosae, AMF form symbiotic associations with a wide range of plant species. Interestingly, there seem to be striking similarities between signalling in rhizobial and arbuscular mycorrhizal symbiosis (reviewed by Hirsch and Kapulnik, 1998). Apart from the effect of plant derived secondary plant compounds (SPC) on the bacterial and the fungal symbiont, SPC (e.g. flavonoids) are accumulated in the roots of the respective host plants during the establishment of both symbioses. Whereas there is some information on the role of SPC in the rhizobial symbiosis, the exact role of SPC during the establishment of the AM symbiosis still remains unclear.

The data about flavonoids in the arbuscular mycorrhizal symbiosis have been extensively reviewed (Vierheilig et al., 1998a). Although there is new information on the effect of various flavonoids on the growth of arbuscular mycorrhizal fungi, and the induction of flavonoids during root colonization by arbuscular mycorrhizal fungi, there is still a debate on the exact role of flavonoids during the formation of the arbuscular mycorrhizal symbiosis. Based on current work, we hypothesize that a fundamental molecular dialogue occurs that regulates not only the early development of AM symbiosis, but also subsequent colonization which has to be balanced for establishment of genuine mycorrhizal symbiosis. In this work some aspects of the signalling and regulation in rhizobial and arbuscular mycorrhizal symbiosis are compared and some possible functions of secondary plant compounds e.g.

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flavonoids, in the mycorrhizal symbiosis are presented. We hope this overview gives some stimulation for further studies on signalling in AM.

2. THE LEGUMES-RHIZOBIA INTERACTION

2.1. Signalling During the Formation of the Rhizobial Symbiosis

Abundant information is available about signalling in the legume-rhizobium interaction. Root exudates of legumes seem to stand at the beginning of a complex signal exchange cascade (Fig. 1). Root exudates contain a wide range of compounds, however, during the formation of the rhizobial symbiosis secondary plant compounds, specifically flavonoids (reviewed by Phillips and Tsai, 1992), play a key role. Flavonoids act as chemoattractants for the rhizobial bacteria as specific inducers of rhizobial nodulation genes (nod-genes), which are involved in the synthesis of lipo-chitooligosaccharide signals, called Nod-factors (recently reviewed by Perret et al., 2000). Nod-factors induce in roots of certain legumes the accumulation of flavonoids resulting in the secretion of more flavonoids by the root, which in turn stimulate further production of Nod-factors by the bacteria (Recourt et al., 1992; Dakora et al., 1993; Schmidt et al., 1994; Bolanos-Vasquez and Werner, 1997). Nod-factors are required for the rhizobial penetration of roots hairs of the host plant. After penetration of the root, the bacterium reaches through an infection thread the root cortex, where dividing cells form a new symbiotic organ, the nitrogen-fixing nodule.

Figure 1. Schematic model of the signalling in the legume-Rhizobium-arbuscular mycorrhizal fungus (AMF) interaction. The legume-Rhizobium interaction (1-4). The legume-Rhizobium-AMF interaction (6-7). Flavonoids are exuded by roots of legumes (1) activating nod-genes in the rhizobial bacteria (2). The bacteria start to produce Nod-factors (3) which are perceived by the legume root and induce the accumulation and exudation of flavonoids by the root (4). The flavonoids again activate nod-genes (5). The changes in the root exudation also seem to enhance root colonization by AMF (7), possibly through a stimulatory effect on hyphal growth of AMF (6).
2.2. Autoregulation of Nodulation

Ample evidence indicates that the rhizobial host plant can control the extent of nodulation (for details see review Caetano-Anollés and Gresshoff, 1991). Once a critical number of nodules has formed on the root system further nodulation is nearly completely suppressed. The formation of nodules is a costly process for the host, so it is not surprising to find that nodulation is controlled by a plant-mediated autoregulatory mechanism. Interestingly, the autoregulatory mechanism is systemic. Inoculation of one half of a split root system with rhizobia strongly reduces nodulation on the other half of the root system, whereas the total number of nodules formed on a host plant remains constant. Little is known about the mechanisms and the signals involved in the local and systemic autoregulation of nodulation.

2.3. The Legume-Rhizobia-AMF Interaction

In many studies an enhanced root colonization by AMF and rhizobia has been reported when co-inoculated (Daft and El-Giahmi, 1974; Cluett and Boucher, 1983; Kawai and Yamamoto, 1986; Pacovsky et al., 1986; Chaturvedi and Singh, 1989; Xie et al., 1995). A more detailed study with soybean mutants which are unable to form nodules (Nod') revealed that root colonization by AMF was even increased when Nod soybeans were inoculated with rhizobia (Xie et al., 1995). These data clearly demonstrated that the stimulatory effect on AM root colonization by rhizobia is not linked to nitrogen fixation but rather to a pre-nodulation event. When soybean plants were co-cultivated with AMF and a rhizobial mutant strain, which was deficient in Nod-factor biosynthesis and thus non-nodulating (Nod') or a rhizobial strain (Nod') with a normal Nod-factor biosynthesis, the rhizobial Nod' strain showed a clearly stimulatory effect on AMF colonization, whereas the rhizobial Nod' strain showed no effect. These results suggested a stimulatory effect of Nod-factors on AM root colonization. However, testing two Nod-factors differing in their ability to induce the secretion of flavonoids in soybean, the Nod-factors could be excluded as the responsible factors for the increased root colonization. Only the Nod-factor enhancing the secretion of flavonoids from roots showed a stimulatory effect on AMF root colonization. The Nod-factor having no effect on flavonoid secretion did not affect AM root colonization (Xie et al., 1995). A similarly enhanced AM colonization was observed when Nod-factors were applied to Lablab purpureus, another leguminous species (Xie et al., 1998). These results suggest that certain flavonoids induced in the signal exchange cascade during rhizobial symbiosis formation do have a stimulatory effect on the establishment of the AM symbiosis (see Fig. 1). The application of several Nod-factor-induced flavonoids from soybean to AMF inoculated soybean plants also enhanced AM root colonization (Xie et al., 1995), which seems to link the enhanced AM colonization in presence of rhizobia to an enhanced root exudation of certain flavonoids. The stimulatory effect of flavonoids on root colonization has been shown in a variety of plants (reviewed Vierheilig et al., 1998a; Fig. 2).

To our knowledge no data exist to show whether these changes in root exudation in rhizobial roots also affect other microorganisms in the soil. Legumes inoculated with rhizobia are less damaged by soil borne pathogens (Chakraborty and Purkayastha, 1984; Chakraborty and Chakraborty, 1989; Tu, 1978). No evidence has been presented so far to explain this protective effect conclusively. However, as flavonoids also exhibit antifungal
Figure 2. Effect of Biochanin A on root colonization of clover and tomato by the AMF, *Glomus mosseae*. Plantlets (10 d old) were transferred into pots containing an autoclaved sand/loam substrate (1:1: v:v) mixed with an inoculum of *G. mosseae* (containing sporocarps, spores, hyphae and colonized root pieces). Biochanin A (dissolved in MeOH; final concentration 0.5%) was applied at time of transplanting and two weeks later. After four weeks roots were harvested and root colonization was determined.

Activities (Van Etten, 1976; Wyman and Van Etten, 1978; Weidenbörner and Jha, 1994; Dakora and Phillips, 1996), changed concentrations of these compounds in root exudates of rhizobial plants could affect soil borne plant pathogens.

These results indicate that changes in the root exudates occur, which not only play a key role during the formation of the rhizobial symbiosis, but which also affect AMF and possibly other soil microorganisms.

3. THE PLANT-AMF INTERACTION

3.1. The Precolonization Stage (Signals from the Plant to the Fungus)

The association between legumes and rhizobia is perhaps the most extensively studied plant-microbe interaction, and the signalling between the host and bacterium is fairly well understood. Little is known, however, about signalling during the formation of the AM symbiosis, although numerous publications exist. Data on the effect of various compounds on hyphal growth and on the accumulation of compounds in mycorrhizal roots are available (recently reviewed by Vierheilig et al., 1998a), however, the significance of these events for the outcome of the symbiosis remains unclear. This lack of understanding is partially due to the difficulty to work with AMF. In contrast to rhizobia, AMF can not be cultured in the
absence of a host plant, thus it can be difficult to distinguish between plant and fungal effects. Most data are available about signals originating from the plant during the precolonization stage, i.e., the phase before root colonization occurs.

Similar to the legume-rhizobia interaction, root exudates appear to be at the beginning of a signal exchange chain between the AM host plant and the AMF (Fig. 3). Root exudates exhibit an attractional effect on the growth of AMF (Gemma and Koske, 1988; Koske, 1982; Koske and Gemma, 1992; Suriyapperuma and Koske, 1995; Vierheilig et al., 1998b). The nature of the attracting compounds is still unidentified. More information is available about the compounds in root exudates affecting AMF growth and spore germination (see review Vierheilig et al., 1998a). In general, under axenic conditions root exudates of AM host plants exhibited a clearly stimulatory effect. In light of the importance of flavonoids exuded by roots during establishment of rhizobial symbiosis, the effects of these compounds on AMF was tested early. In 1989 (Gianinazzi-Pearson et al.) the first report was published on AM hyphal growth stimulation by the flavonoids hesperetin, apigenin and naringenin. A short time later a synergistic stimulatory effect of flavonoids and enhanced CO₂ concentration on AM hyphal growth was reported (Bécard et al., 1992; Chabot et al., 1992; Poulin et al., 1993). Thereafter many flavonoids and other SPC were tested for their effects on axenic growth of AMF, some exhibiting a clearly stimulatory effect and some exhibiting an inhibitory or no effect at all (see Vierheilig et al., 1998a).

The effect of root exudates and flavonoids was also tested on root colonization by AMF. Interestingly application of root exudates of non-mycorrhizal plants (Tawaraya et al., 1998)
or of certain flavonoids to AMF inoculated plants enhanced root colonization (see review by Vierheilig et al., 1998a; Fig. 2).

Although the data on the stimulatory effect of root exudates and flavonoids on axenic growth and root colonization by AMF are certainly interesting, their biological relevance to the outcome of the mycorrhizal symbiosis still has to be demonstrated. Bécard et al. (1995) recently questioned an essential role of flavonoids during the establishment of the AM association.

### 3.2. The Precolonization Stage (Signals from the Fungus to the Plant)

To our knowledge, due to the inability to culture AMF axenically, no direct evidence of the presence of fungal derived signals has been reported as yet. However, there is some support for the view that AMF release signalling compounds which can be perceived by the root and which are probably essential for root colonization by AMF.

In general, plants in the Brassicaceae family are nonhosts for AMF (Harley and Harley, 1987). The inability of these plants to form the symbiosis is characterized by the absence of fungal structures in the root or even on the root surface. In roots of Brassicacea inoculated with AMF changes of the β-1,3-glucanase and chitinase activity (Vierheilig et al., 1994), and changes of the glucosinolate levels could be observed (Vierheilig et al., 2000a). The biological function of these changes is not clear, however, these observations point to the presence of AM fungal derived signals sensed by plants. The non-specific character of these factors is suggested since even AM nonhost plants seem to sense the presence of AMF. A so-called "Myc-factor" (analogous to the Nod-factor known from rhizobia) recently has been proposed by Albrecht et al. (1998) and Bilou et al. (1999). Albrecht et al. (1998) found a similar induction of early noduline genes in pea roots by an AMF and by rhizobial Nod-factors, and Bilou et al. (1999) reported a similar suppression of salicylic acid in pea by Nod-factors and AMF.

Xie et al. (1999) detected a specific chitinase isoform in soybean roots, which was only induced in mycorrhizal roots and in roots treated with Nod-factors, but not in non-mycorrhizal untreated roots. Interestingly, a chitinase isoform found in mycorrhizal clover roots was also induced under sterile conditions when AM mycelium was applied to clover roots (C. Albrecht and H. Vierheilig, unpublished results), pointing to a certain functional similarity of signals in the fungal mycelium with Nod-factors. This would not be surprising as Nod-factors share structural characteristics with fungal chitin elicitors. However, direct evidence for the presence of "Myc-factor" is still missing.

### 3.3. Penetration and Root Colonization

After the AMF has reached the root, hyphal growth on the root surface and the formation of penetration structures, the appressoria, occur. Finally the fungus penetrates the root, forming internal hyphae and arbuscules. The molecular mechanisms controlling the penetration, the establishment and the functioning of the symbiosis are still unclear. SPC are thought to play a role during these events, as the accumulation of several SPC has been reported in mycorrhizal plants (see review Vierheilig et al., 1998a).

The most extensive information on the accumulation pattern of SPC in mycorrhizal plants is available about isoprenoid cyclohexenone derivatives (CD). Several studies of roots
of many members of the Poaceae colonized by AMF showed the widespread occurrence of glycosylated C13 cyclohexenone derivatives in this plant family (Maier et al., 1995, 1997; Peipp et al., 1997; Vierheilig et al., 2000b). Recently CD have been detected in mycorrhizal roots of Nicotiana tabacum (Maier et al., 1999, 2000) and Lycopersicon esculentum (Maier et al., 2000), both members of the Solanaceae, showing that their occurrence is not restricted to the Poaceae. CD accumulation is specifically induced by AMF, but not by root pathogens or other endophytes (Maier et al., 1997), and is directly correlated with the degree of mycorrhization (Maier et al., 1995). Various AMF from different species or genera induce qualitatively similar CD accumulation patterns (Vierheilig et al., 2000b). Although there is some information on the accumulation of CD in mycorrhizal roots, nothing is known yet about the function of these compounds in mycorrhiza.

Fewer studies exist about the accumulation of flavonoids, another group of SPC, in mycorrhizal roots. However, abundant data are published about their effect, and thus their possible function, on AMF (Vierheilig et al., 1998).

A number of flavonoids could be identified in mycorrhizal roots (Harrison et al., 1993; Volpin et al., 1994). In fully colonized Medicago sativa plants, increased levels of 4',7-dihydroxyflavone, formononetin, coumestrol and daidzein could be detected (Harrison et al., 1993). In roots, where root colonization was halted at the appressoria formation stage 4',7-dihydroxyflavone and coumestrol was not detectable, but formononetin and daidzein accumulated. These observations pointed to accumulation of certain compounds at different stages of the root colonization process and could indicate their function at different steps during the formation of the symbiosis.

The similarity between the rhizobial and the mycorrhizal symbiosis is striking. In both symbioses SPC are accumulated in the roots. However, where in rhizobial symbiosis flavonoids accumulated are released by the root interacting with the bacterium, the probable function of flavonoids and CD in mycorrhizal roots is speculative. It is interesting to hypothesize how secretion of these SPC by the mycorrhizal root affects further mycorrhization.

4. THE PLANT-AMF-AMF INTERACTION

4.1. Is There an Autoregulation of Mycorrhization?

The AM association is not a static event. After a first colonization of a root by an AMF, the same AMF or other AMF can colonize the same root or other parts of the root system of the same plant. From the plant's perspective, the development of the mycorrhizal association is a costly process. Thus, after a critical degree of root colonization is reached, suppression of further root colonization by AMF is likely to occur in order to reduce the cost of symbiosis for the plant as a carbon source. Such an autoregulatory mechanism is known in rhizobial symbiosis (see 2.2 and Caetano-Anollés and Gresshoff, 1991).

Results on this hypothesis have been recently published. In several studies simultaneous inoculation with different AMF reduced root colonization of each individual AMF (Daft and Hogarth, 1983; Wilson, 1984; Hepper et al., 1988; Pearson et al., 1993, 1994). However, under these conditions a faster root colonizer could simply dominate a slower root colonizer by occupying the biological niche in the root, thus reducing root colonization by the slow colonizer. To address this possibility, the effect of precolonization by an AMF on
further root colonization by AMF was tested in a recent experiment. One side of a split root system of barley plants was inoculated with *G. mosseae*, *G. intraradices* or *Gigaspora rosea*. When the fungi extensively colonized the roots, the other side of the split root system was inoculated with *G. mosseae*. The results showed a clear reduction of subsequent root colonization once the barley plants were precolonized with any of the tested AMF (Vierheilig et al., 2000c), indicating a systemic suppression of further root colonization of plants colonized by an AMF. The effect was also observed when *G. mosseae* precolonized plants were subsequently inoculated with *G. mosseae*, pointing to an autosuppression of further root colonization by the same fungus (Vierheilig et al., 2000c).

Pearson et al. (1993) suggested several mechanisms to explain the reduction of AMF colonization in roots colonized by another AMF: i) a competition of different AMF for carbohydrates, ii) a mechanism involving the improved P-status of AMF colonized plants, iii) an altered accumulation of compounds in mycorrhizal roots affecting AMF.

A comparison of the root growth in mycorrhizal and non-mycorrhizal split-root systems and the observation that subsequent root colonization by *G. mosseae* was suppressed in *G. mosseae* precolonized plants makes it rather unlikely that the observed suppression in mycorrhizal plants was due to a simple competition for carbohydrates between AMF (Vierheilig et al., 2000c). Moreover, P-application experiments in split root systems demonstrated that improved P-status and the suppressional effect are not linked in mycorrhizal plants (Vierheilig et al., 2000d).

Changes in the accumulation pattern of flavonoids resulting in an altered root exudation have been reported from the rhizobial symbiosis in legumes (see 2.1). Moreover, it has been reported that changes in the root exudation pattern during the formation of the rhizobial symbiosis in legumes can affect root colonization by AMF (Xie et al., 1995; 1998). Thus it is reasonable to speculate that root exudates of mycorrhizal plants exhibit a different effect on AMF than root exudates of non-mycorrhizal plants. Pinior et al. (1999) showed that root exudates of cucumber plants colonized by the AMF *G. mosseae*, *G. intraradices* or *Gl. rosea* applied to cucumber plants inoculated by *G. mosseae* exhibited a different effect on root colonization than root exudates of non-mycorrhizal cucumber plants. All root exudates from mycorrhizal plants showed a clearly inhibitory effect on root colonization by *G. mosseae*, and root exudates of non-mycorrhizal cucumber plants slightly stimulated root colonization. The inhibitory effect of mycorrhizal root exudates on root colonization was similar, independent of the root colonizing fungus. Root exudates from plants colonized by *G. mosseae* also inhibited root colonization by *G. mosseae*. A similar autosuppression effect has been observed in split root systems as discussed above (Vierheilig et al., 2000c).

From the data presented an altered host status of mycorrhizal and non-mycorrhizal roots is suggested. Once the roots are mycorrhized, further root colonization by AMF seems suppressed, indicating an autoregulation of the mycorrhization. Changes in the root exudation pattern of mycorrhizal roots seem to be at least partially involved in this suppression.

4.2. Which Compounds Might Be Involved in the Autoregulation of Mycorrhization?

Several SPC are accumulated after root colonization by AMF. The importance of these SPC for the outcome of the AM symbiosis is still unclear. Possibly, the role of SPC, e.g.,
flavonoids, changes during the formation of the mycorrhizal association. Initially, SPC exuded by the root stimulate and attract AMF hyphal growth. Subsequently, AMF-derived signals ("Myc-factors") induce the accumulation and secretion of certain SPC before a cell-to-cell contact and/or at the moment of the appressoria formation, thus stimulating further root colonization. Finally, the fungus penetrates the root, forming its intraradical structures and inducing the accumulation of SPC which are subsequently released into the rhizosphere, thus suppressing further root colonization.

The stimulation of AMF by SPC found in root exudates of non-mycorrhizal plants is fairly well documented (see Vierheilig et al., 1998a). However, there is little evidence to support the other possible functions of SPC proposed in our model, that different flavonoids act and accumulate prior or during different stages of the symbiosis. Although there are indications for the presence of "Myc-factors" (see 3.2), no data are available on the accumulation of SPC induced by these fungus derived signals. Harrison and Dixon (1993) found that some flavonoids are accumulated at the cell-to-cell stage (formononetin and daidzein), before the plant-AMF association is established, whereas others are not (4',7-dihydroxy-flavone and coumestrol). While 4',7-dihydroxy-flavone, formononetin, coumestrol and daidzein are known to stimulate spore germination or hyphal growth of AMF (see review by Vierheilig et al., 1998a), the phytoalexine medicarpin, also accumulated in mycorrhizal Medicago roots, possesses antifungal activity toward pathogenic fungi (Higgins, 1978), thus possibly playing a different role in the symbiosis than the other compounds accumulated. Flavonoids, depending on their nature and concentration, can stimulate fungal growth or exhibit antifungal activity (Van Etten, 1976; Wyman and Van Etten, 1978; Weidenbörner and Jha, 1994; Dakora and Phillips, 1996).

As yet, there are no available data that compounds accumulated in mycorrhizal roots are secreted by the root. However, root exudates of mycorrhizal plants suppress root colonization by AMF (Pinior et al., 1999), suggesting the presence of inhibitory compounds. It is interesting to note that different concentrations of phenolic compounds applied to AMF-inoculated plants can exhibit different effects. Low concentrations of some phenolics can stimulate root colonization, while higher concentrations can reduce root colonization (Fries et al., 1997). Thus, it is possible that the accumulation of SPC occurring after root colonization by AMF triggers an enhanced exudation of these compounds in the rhizosphere and affects further root colonization.

The possible role of phenolics accumulated in mycorrhizal roots in the regulation of mycorrhization is also confirmed by results with ectomycorrhizal fungi. Feugley et al. (1999) reported that the accumulation of phenolic compounds after root colonization by an ectomycorrhizal fungus limited further formation of the Hartig net to the outer layer of the root cortex.

Until now a role of phenolics, specifically flavonoids, has been proposed only for the formation of the symbiotic association, however, we suggest that different phenolics or different concentrations of phenolics might play different roles during different stages of the AM symbiosis.

Some studies with SPC involved in the regulation of root colonization were conducted in the Gramineae. CD are SPC specifically induced by AMF (Maier et al., 1997), and application of the CD, blumenin, to AMF inoculated plants has been shown to reduce root colonization (Fester et al., 1999).

Vierheilig et al. (2000c) reported that in a split root system precolonization by an AMF
on one side suppressed further colonization on the other side. The mechanisms of this suppression are still unknown, however, the accumulation of CD in a mycorrhizal plant might be involved. Studying the accumulation of several CD in split root systems of barley revealed that CD are only accumulated in mycorrhizal roots, but never in non-mycorrhizal roots of a mycorrhizal plant (Vierheilig et al., 2000d). This clearly demonstrated that CD are not systemically induced, and thus can not be involved in the systemic autoregulation of mycorrhization first reported by Vierheilig et al. (2000c). However, a local effect of CD on mycorrhization can not be excluded based on the results presented, although, the lack of antifungal activity of CD on the growth of the fungus *Cladosporium cucumerinum* (Fester et al., 1999) makes a simple growth inhibitory effect on AMF rather unlikely.

The results presented indicate that SPC are involved during different stages of the formation of the AM association. We attempted to consider these results with the possibility that SPC are involved in the regulation of mycorrhization in mycorrhizal plants, however, clear proofs for such a role are still lacking.

4.3. Possible Signalling Compounds in the Autoregulation of Mycorrhization

In systemic acquired resistance (SAR) pre-infection of a plant with a pathogen can result in an enhanced resistance in other parts of the plant to the same or related pathogens (recently reviewed by Sticher et al., 1997). Salicylic (SA) and jasmonic acid (JA), two of the compounds playing a key role in SAR, have been extensively studied (reviewed by Malamy and Klessig, 1992; Pieterse and van Loon, 1999), however, little information is available on either compound in AM (recently reviewed by Ludwig-Müller, 2000).

Transgenic *NahG* plants have been a valuable tool to study SA mediated plant mechanisms. *NahG* plants express the bacterial *nahG* gene, which encodes the enzyme salicylate hydroxylase that inactivates SA. Thus SA is not accumulated in *NahG* plants (Gaffney et al., 1993). Inoculation of *NahG* tobacco plants with AMF resulted in accelerated root colonization, compared with wild type tobacco plants (J. M. Garrido-Garcia and H. Vierheilig, unpublished results), indicating that SA levels in roots can affect root colonization by AMF. Measurements of the SA accumulation in plants inoculated with AMF also indicated that SA is involved in the susceptibility of plants to AMF. In pea mutants (*Myc* plants), which are unable to form the AM symbiosis, the SA accumulation was enhanced, whereas in *Myc* peas the SA accumulation was low (Biliou et al., 1999) or only transient (Biliou et al., 2000a, 2000b) when compared to the *Myc* pea mutants.

These observations suggest a role of SA in the regulation of root colonization by AMF. However, whether enhanced SA levels are the symptom or the cause of reduced colonization still has to be investigated.

Even less data are available about the SAR signalling compound JA in AM (reviewed by Ludwig-Müller, 2000). Recent experiments show that whereas an application of SA to the shoot of cucumber plants shows no effect on root colonization by AMF, while an application of JA to the shoot drastically reduces root colonization (control root colonization 30±4 %, JA treated root colonization 0.5±0.3%) (J. Ludwig-Müller and H. Vierheilig, unpublished results). Moreover, JA is extensively accumulated in mycorrhizal roots and even in non-mycorrhizal roots of a split-root system of mycorrhizal plants, in general, at levels higher than in roots of non-mycorrhizal plants (O. Miersch and H. Vierheilig, unpublished results)(Table 1). These results may indicate an involvement of JA not only in
the local, but also in the systemic suppression of further root colonization by fungi in mycorrhizal plants. From the results available on SA and JA in AM, their importance in the autoregulation of mycorrhization, similar to their role in SAR, cannot be excluded. However, their exact function in local and systemic bioprotective and autoregulatory mechanisms in mycorrhizal plants still has to be elucidated.

**Table 1.** Accumulation of jasmonic acid (JA) in a split root system\(^a\) of non-mycorrhizal (Plants 1 to 3) and of mycorrhizal cucumber plants (Plants 4 to 7). In mycorrhizal plants one half of the split root system was mycorrhizal (+M) and the other half was non-mycorrhizal (-M)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Root colonization (%)</th>
<th>JA (pmol/g FW)</th>
<th>JA (means ± s.e.)</th>
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</thead>
<tbody>
<tr>
<td>Plant 1  non-mycorrhizal plant</td>
<td>0</td>
<td>314</td>
<td>non-mycorrhizal plants 398 ± 106</td>
</tr>
<tr>
<td>Plant 2  non-mycorrhizal plant</td>
<td>0</td>
<td>364</td>
<td></td>
</tr>
<tr>
<td>Plant 3  non-mycorrhizal plant</td>
<td>0</td>
<td>517</td>
<td></td>
</tr>
<tr>
<td>Plant 4  mycorrhizal plant Side -M</td>
<td>0</td>
<td>832</td>
<td>mycorrhizal plants Side -M 627 ± 145</td>
</tr>
<tr>
<td>Plant 4  mycorrhizal plant Side +M</td>
<td>29</td>
<td>1943</td>
<td></td>
</tr>
<tr>
<td>Plant 5  mycorrhizal plant Side -M</td>
<td>0</td>
<td>568</td>
<td></td>
</tr>
<tr>
<td>Plant 5  mycorrhizal plant Side +M</td>
<td>41</td>
<td>10480</td>
<td></td>
</tr>
<tr>
<td>Plant 6  mycorrhizal plant Side -M</td>
<td>0</td>
<td>496</td>
<td></td>
</tr>
<tr>
<td>Plant 6  mycorrhizal plant Side +M</td>
<td>64</td>
<td>2178</td>
<td>mycorrhizal plants Side +M 5624 ± 4249</td>
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<tr>
<td>Plant 7  mycorrhizal plant Side -M</td>
<td>0</td>
<td>613</td>
<td></td>
</tr>
<tr>
<td>Plant 7  mycorrhizal plant Side +M</td>
<td>49</td>
<td>7896</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Five days (d) old cucumber plants were excised of the main root and grown for 5 d. Thereafter plants were transferred into the compartment system described by Vierheilig et al. (2000c) in order to colonize the split root systems by the AM fungus *Glomus mosseae* (BEG 12). Control plants were not inoculated. Sixteen days after the inoculation plants were harvested and the roots were stored at -20°C. The accumulation of JA was determined in lyophilized roots (Miersch and Wasternack 2000). Root colonization was determined as described by Vierheilig et al. (1998c).

5. AUTOREGULATION OF MYCORRHIZATION AND ENHANCED RESISTANCE TOWARD SOIL BORNE FUNGAL PATHOGENS IN MYCORRHIZAL PLANTS: ONE MECHANISM, TWO SYMPTOMS?

In many studies the enhanced resistance of mycorrhizal plants toward soil borne fungal pathogens has been reported (Dehne, 1982). The mechanisms involved in the resistance
have not been clearly identified (Azcon-Aguilar and Barea, 1996; Hooker et al., 1994; Morandi, 1996; St.-Arnaud et al., 1995). Although there is no experimental evidence yet for a common mechanism of autoregulation and enhanced resistance, it is tempting to speculate that they share certain features.

An indication for a common mechanism is the effect of root exudates of mycorrhizal plants on pathogens and AMF. The AMF non-host plant *Dianthus caryophyllus* co-cultured with the mycorrhizal AMF host plant *Tagetes patula* reduced disease development by *Fusarium oxysporum* in *D. caryophyllus* (St.-Arnaud et al., 1997) suggesting the presence of bioprotective compounds released by the roots of the mycorrhizal plant. This observation is confirmed by earlier data presented by Caron et al. (1986), Meyer and Linderman (1986) and Bansal and Mukerji (1994), reporting suppression of pathogenic fungi in the soil around mycorrhizal roots.

More detailed studies on root exudates of mycorrhizal and non-mycorrhizal plants revealed some interesting details. In experiments in vitro, root exudates of non-mycorrhizal plants exhibited a clearly stimulatory effect on the sporulation of the pathogenic fungus *Phytophthora fragariae* (Norman and Hooker, 2000), and the hyphal growth of AMF (Pinior et al., 1999). However, when root exudates of mycorrhizal plants were collected no stimulation could be observed, pointing to the absence of a stimulatory compound/s or the presence of a inhibitory compound/s in the root exudates of mycorrhizal plants.

Root exudates of mycorrhizal plants seemed not only to affect fungal sporulation and growth, but also reduced the susceptibility of roots to infection by fungi. Working with tomato plants and the soil borne pathogen *P. parasitica*, Vigo et al. (2000) explained a decreased susceptibility of mycorrhizal roots to pathogens by a reduction of infection loci. A similar mechanism could explain the reduced root colonization by AMF in cucumber root, treated with root exudates of mycorrhizal cucumber plants, as these exudates exhibited no direct inhibitory effect on AMF (Pinior et al., 1999).

To summarize, the similar effect of root exudates of mycorrhizal plants on AMF and soil borne pathogens could be explained by changes in the exudates, not only reducing fungal growth, but also reducing possible infection sites on the host roots. Whether the same compounds in the exudates of mycorrhizal roots do affect pathogens and AMF needs further investigation.

Further evidence for a possibly common mechanism is the systemic effect observed in autoregulation and in plant resistance. In several studies a systemic suppressive effect of AMF root colonization on soil borne fungal pathogen infection has been demonstrated (Davis and Menge, 1980; Rosendahl, 1985; Cordier et al., 1998). The suppression of further root colonization by AMF in already mycorrhized plants also appears to be regulated systemically (Vierheilig et al., 2000c, 2000d). The signals that control the systemic autoregulatory mechanism of mycorrhization and the enhanced systemic resistance in mycorrhizal plants are not yet known. A common signalling mechanism seems possible (see 4).

Another indication for a common mechanism in autoregulation and enhanced resistance is that only a well established AM symbiosis can protect plants against soil borne pathogens. In several reports the requirement of extensive root colonization by AMF for enhanced resistance has been presented (Cordier et al., 1996, 1998; Caron et al., 1986; Bärttschi et al., 1981) and recently it was suggested that a bioprotective effect depends on a fully developed symbiosis characterised by the presence of arbuscules (Slezack et al., 2000). The recently reported systemic autoregulatory mechanisms suppressing further root
colonization by AMF in already mycorrhizal plants also seems to depend on the intensity of prior mycorrhization (Vierheilig et al., 2000c, 2000d). High pre-colonization on one side of a split root system results in high reduction of root colonization on the other side, and low pre-colonization results in low reduction of further root colonization (Vierheilig and Piché unpublished results). Thus both phenomena, autoregulation and enhanced resistance, seem to depend on an extensive root colonization by AMF.

Although the presented data can be interpreted differently, it is reasonable to propose "one mechanism, two symptoms" for autoregulation and bioprotection in mycorrhizal plants. It is possible that mycorrhizal plants, while trying to limit the cost of the AM symbiosis, also acquire bioprotection against pathogenic fungi. It is plausible that an already mycorrhized plant develops only a single mechanism to repulse further colonization by fungi, not discriminating between AM fungi and soil-borne pathogenic fungi. Although this hypothesis is very exciting, the data available so far can only offer indications for its validity. However, clear proof is still lacking. Further studies are needed to elucidate the exact mechanism for autoregulation of mycorrhization and enhanced resistance toward soil-borne pathogens in mycorrhizal plants.

6. CONCLUSION

The data presented show evidence for the presence of signalling compounds during AM symbiosis. While abundant data exist of the exact nature of certain signals during rhizobial symbiosis, no conclusive evidence exists concerning signalling in mycorrhizal association.

It is interesting to find that root exudates and SPC, such as flavonoids, found in root exudates can stimulate hyphal growth and are accumulated in mycorrhizal plants. Although some of these observations are reminiscent of the rhizobial symbiosis and thus the idea that they also might play an important role in AM is tempting, it can not be concluded at present that these compounds really have an essential function in the AM symbiosis.

SPC, such as flavonoids, have been studied mainly whether they are essential for the outcome of the symbiosis. The necessity of flavonoids for the establishment of AM has been previously questioned (Bécard et al., 1995). However, it is tempting to speculate that flavonoids, irrespective of their possible involvement during the formation of AM, play a role in mycorrhizal autoregulation and bioprotection.

It is surprising that the majority of studies on the bioprotective effect of mycorrhiza concentrate on the processes after root colonization and little information is available on the character of the changes occurring in the mycorrhizosphere. While defense mechanisms for bioprotection in the mycorrhizal root may be difficult to identify, identification of the mechanisms producing the changes in the mycorrhizosphere may be easier. Compounds in root exudates of mycorrhizal plants and/or extraradical mycelium of AMF, responsible for the observed effects should not be too difficult to identify. Key to these studies is an understanding of the mode of action of these compounds, involving the impact on the microbial population in the soil, including AMF.

In this work we extended current knowledge about signalling in AM symbiosis and compared it with signalling mechanisms in rhizobial symbiosis. We proposed some new hypotheses regarding the autoregulation of mycorrhization in connection with the bioprotective effects observed in mycorrhizal plants. Future studies will be focused on
validating the hypotheses presented. Recent studies do show, however, exciting findings corroborating different aspects of these hypotheses.

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