

# Regulatory mechanisms during the plant – arbuscular mycorrhizal fungus interaction<sup>1</sup>

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**Abstract:** Abundant data are available on some aspects of the arbuscular mycorrhizal symbiosis, for example, plant nutrition, but because of difficulties immanent to arbuscular mycorrhizal fungi, such as the inability to culture them axenically, the relatively long time it takes to achieve root colonization, and the simultaneous presence of different morphologic stages of the fungus in the root, less information is accumulated on other aspects such as the regulation of mycorrhization. Regulatory processes in the plant – arbuscular mycorrhizal fungus interaction start before root colonization by the fungus and even before a direct physical contact between the host and the fungal symbiont. Some of the signals exchanged are still a matter of debate and will be discussed further on. After the penetration of the root by the fungus, depending on the developmental stage of the arbuscular mycorrhizal association (e.g., early or mature), a range of plant responses is activated. The possible function of several plant responses in the regulation of mycorrhization is discussed.

*Key words:* arbuscular mycorrhiza, Glomales, autoregulation, flavonoid, recognition, root exudates.

**Résumé :** Il existe une abondance de données sur certains aspects de la symbiose mycorhizienne arbusculaire, par exemple, nutrition de la plante, mais à cause de difficultés inhérentes aux champignons mycorhiziens arbusculaires, telles que l'incapacité de les cultiver axéniquement, la durée relativement longue nécessaire pour la colonisation racinaire et la présence simultanée de différents stades morphologiques du champignon dans la racine, il y a beaucoup moins d'information disponible sur certains aspects tel que la régulation de la mycorhization. Les processus de régulation dans les interactions entre la plante et le champignon mycorhizien arbusculaire commencent avant la colonisation racinaire par le champignon et même avant que le contact physique entre l'hôte et le champignon symbiotique ne soit établi. Certains des signaux échangés font toujours l'objet de controverses et l'auteur en discute. Après la pénétration de la racine par le champignon, selon le stade de développement de l'association mycorhizienne arbusculaire (p. ex., hâtif ou mature), on observe l'activation d'un ensemble de réactions dans la plante. L'auteur discute la fonction possible de plusieurs réactions de la plante dans la régulation de la mycorhization.

*Mots clés :* mycorhize arbusculaire, Glomales, autorégulation, flavonoïdes, reconnaissance, exsudats racinaires.

[Traduit par la Rédaction]

## Introduction

Arbuscular mycorrhizal (AM) fungi are symbiotic soil fungi that colonize roots of about 80% of vascular plants. The mycorrhizal symbiosis enhances the growth and survival of numerous plant species (Smith and Read 1997). The complex cellular relationship between the host roots and AM fungi requires a continuous exchange of signals, which in turn affects the regulation of genes whose products participate in metabolic and structural changes that lead to the symbiosis (Gianinazzi-Pearson 1996). There are several recent reports reviewing in detail the molecular and cellular aspects during the establishment of the AM symbiosis, starting from early events such as the exudation of signalling

compounds by the root, over the appressoria formation, the penetration of the root, to the establishment of the fungus in the root characterized by the arbuscule formation (Harrison 1999; García-Garrido and Ocampo 2002; Pozo et al. 2002).

In this work, based on the aforementioned information and recent data, new hypotheses on regulatory mechanisms in the AM association such as (i) the presence of signals released by AM fungi, which are perceived by the root before appressoria; (ii) the regulation of AM fungal root penetration through the accumulation of secondary plant compounds depending on the P status of the host plant; (iii) regulatory events in the mature AM association; (iv) the regulation of further mycorrhization in already mycorrhizal plants compared with the autoregulation of nodulation; and (v) the role of an altered root exudation in mycorrhizal plants compared with noncolonized plants, are discussed.

## The perception of the AM fungus by the plant before appressoria formation

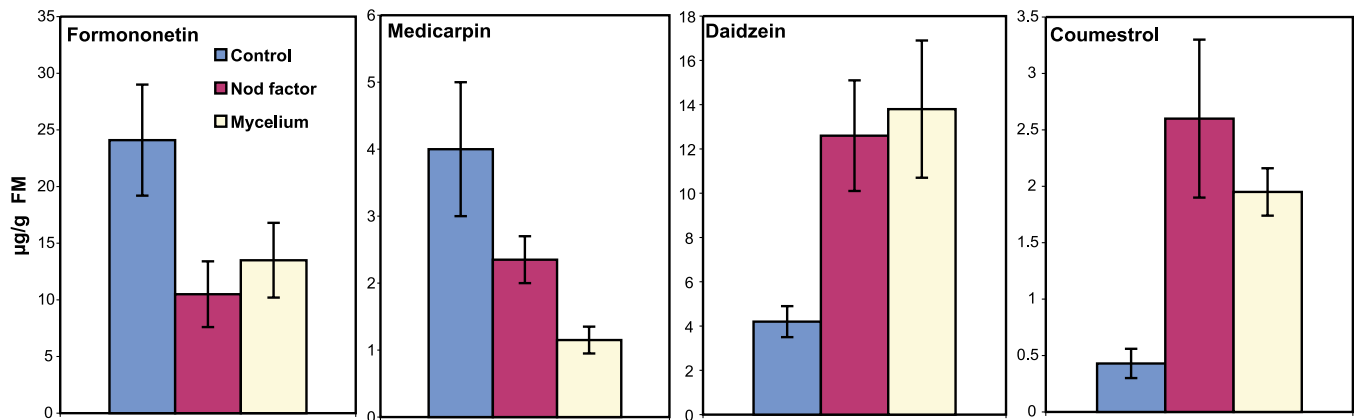
More and more data are accumulated about the presence of AM fungal signals perceived by the root before penetration or even appressoria formation (Salzer and Boller 2000;

Received 7 October 2003. Published on the NRC Research Press Web site at <http://canjbot.nrc.ca> on 1 September 2004.

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<sup>1</sup>This article is one of a selection of papers published in the Special Issue on Mycorrhizae and was presented at the Fourth International Conference on Mycorrhizae.

**Fig. 1.** Flavonoid accumulation in *Medicago sativa* roots 50 h after the application of a mixture of hyphae and spores of the arbuscular mycorrhizal fungus *Glomus intraradices* (for details see Larose et al. 2002) or Nod factor purified from *Sinorhizobium meliloti* (for details see Catford et al. 2003). Data are the means  $\pm$  SD of four replicates from four individual plants (C. Staehelin, G. Larose, and H. Vierheilig, unpublished data).



Vierheilig and Piché 2002). Nonhost plants are an interesting system in which to study the presence of AM fungal signals. Roots of plants of AM nonhost families, such as the *Brassicaceae*, are not penetrated by AM fungi, and in general no AM fungal structures are attached to the root surface; thus, any changes in the root in the presence of AM fungi should be due to diffusible AM-derived signals. In roots of *Brassicaceae* plants the activities of the two hydrolases  $\beta$ -1,3-glucanase and chitinase (Vierheilig et al. 1994) and glucosinolate levels (Vierheilig et al. 2000a) were altered when plants were inoculated with AM fungi, and inoculation of the AM nonhost plant *Salsola kali* with an AM fungus initiated resistance events in the roots (Allen et al. 1989).

Experiments with AM host plants, which, because of their high P status, are not colonized by AM fungi, also indicate the presence of AM fungal signals perceived by the root before penetration. In P-treated alfalfa plants, with no sign of root colonization by the inoculated AM fungus *Glomus intraradices*, formononetin levels were increased (Volpin et al. 1994).

To study the release of elicitor factors by AM fungal tissue, roots have been challenged with spores and hyphal fragments, and changes in the roots were studied. Interestingly, in the presence of a spore extract of *G. intraradices* in tomato roots transformed with Ri T-DNA, new polypeptides appeared (Simoneau et al. 1994). Application of spores and hyphal fragments of *G. intraradices* to roots of alfalfa resulted in alterations of the accumulation pattern of several flavonoids (Fig. 1; Larose et al. 2002).

The biological function of these changes remains unclear; however, they point towards the presence of signals derived from AM fungi sensed by plants before appressoria formation. The nonspecific character of these factors is suggested, as even AM nonhost plants seem to sense the presence of an AM fungus. A so-called Myc-factor (analog to the Nod factor known from rhizobia) recently has been proposed by Albrecht et al. (1998) and Blilou et al. (1999). Albrecht et al. (1998) found a similar induction of early nodulin genes in pea roots by an AM fungus and by rhizobial Nod factors, and Blilou et al. (1999) reported a similar suppression of salicylic acid in pea by Nod factors and AM fungi.

A certain functional similarity between signals from the AM fungal mycelium or Nod factors would not be surprising, as Nod factors, with their chitin backbone, share structural characteristics with fungal chitin elicitors. This seems to be confirmed by a similar accumulation pattern of a number of flavonoids in alfalfa roots treated with AM fungal tissue or Nod factors (Fig. 1). Nod-factor application as well as the application of AM fungal tissue results in increased daidzein and coumestrol levels and in reduced formononetin and medicarpin levels.

Recently, Kosuta and coworkers (2003) showed that Nod factors and signals derived from AM fungi, although they might share certain similarities, are not identical. A diffusible factor from different AM fungi induces symbiosis-specific MtENOD11 expression in roots of *Medicago truncatula*; however, the induction pattern was different compared with roots treated with Nod factor. The diffusible AM fungal factor seems specific to AM fungi, as no MtENOD11 induction was observed with several fungal pathogens.

Owing to the inability to culture AM fungi axenically and the lack of a clear biological indicator in the plant, studies on the presence of a possible Myc-factor are difficult; however, the Myc-factor is more and more a matter of intensive studies, and recent studies give promising new insight in understanding early signaling events leading to mycorrhization (Ben Amor et al. 2003; Kosuta et al. 2003).

## Root colonization by AM fungi

### The regulation of AM fungal root penetration by the host plant

Depending on P availability, the AM fungal host status of a plant can vary. In general, in plants with a high P status root colonization is reduced or absent, whereas low P levels enhance root colonization. The exact mechanisms involved are still unknown; however, in several works a decrease of the cell membrane permeability in plants grown at high P conditions, resulting in a lower leakage of amino acids and sugars, has been linked with a lower root colonization (see Smith and Read 1997).

**Table 1.** Precolonization events between host plants with a low P status and arbuscular mycorrhizal (AM) fungi.

	Responses by plants and (or) AM fungus	Reference
Fungal responses to plant signals	Stimulation of hyphal growth and branching by root exudates	Elias and Safir 1987; Tawaraya et al. 1996, 1998; Nagahashi et al. 1996
	Root exudates stimulate root colonization	Tawaraya et al. 1998
	Stimulation of root colonization through compounds accumulated in roots	Akiyama et al. 2002
Plant and fungal responses	When exposed to AM-fungal-derived signals in roots of <i>Medicago sativa</i> , accumulation of compounds (e.g., coumestrol, daidzein) that are stimulatory to hyphal growth and root colonization	Larose et al. 2002; Morandi et al. 1992; Xie et al. 1995

**Table 2.** Precolonization events between host plants with a high P status and arbuscular mycorrhizal (AM) fungi.

	Responses by plants and (or) AM fungus	Reference
Fungal responses to plant signals	Reduced stimulation of spore germination and hyphal growth by root exudates	Tawaraya et al. 1996; Elias and Safir 1987; Nagahashi et al. 1996
	Root exudates do not stimulate root colonization	Tawaraya et al. 1998
	No accumulation of compounds stimulatory for root colonization in roots	Akiyama et al. 2002
Plant responses to fungal signals	When inoculated with AM fungi, accumulation of compounds inhibitory for AM fungal spore germination and hyphal growth	Guenoune et al. 2001

There seems to be a clear difference in the biological activity of root exudates from high-P and low-P plants (Tables 1 and 2). Whereas root exudates of low-P plants stimulate hyphal branching, hyphal growth (Elias and Safir 1987; Tawaraya et al. 1996; Nagahashi et al. 1996; and many others, see review of Vierheilig et al. 1998a), and root colonization (Tawaraya et al. 1998; Pinior et al. 1999), root exudates of high-P plants show a reduced stimulation of hyphal growth (Elias and Safir 1987; Tawaraya et al. 1996) and no stimulation of root colonization (Tawaraya et al. 1998).

In recent studies, flavonoid levels varying with the P status of the plant have been suggested to play a major regulatory role at this stage of mycorrhization. The accumulation pattern of flavonoids in plants seems to depend on their P status. In alfalfa plants with a high P status after inoculation with the AM fungus *G. intraradices*, medicarpin was highly accumulated, although no fungal structures were present within the root. Medicarpin has been reported to exhibit a strong inhibitory effect on spore germination and hyphal growth of the AM fungus (Guenoune et al. 2001) and is only weakly accumulated in mycorrhizal roots with a low P status. Thus, Guenoune et al. (2001) suggested that the inhibition of colonization of alfalfa plants treated with high P is due to the accumulation of AM fungal inhibitory compounds such as medicarpin.

Interestingly, Larose et al. (2002) reported that in alfalfa plants with a low P status, signals derived from an AM fungus are perceived by the root before appressoria formation, reacting with alterations of its flavonoid pattern. In the presence of fungal tissue of *G. intraradices*, coumestrol and daidzein, both compounds stimulatory to hyphal growth and (or) root colonization (Morandi et al. 1992; Xie et al. 1995), were accumulated at higher levels, whereas the levels of formononetin and the AM fungal inhibitory compound medicarpin (Guenoune et al. 2001) were reduced (Larose et al. 2002; and see Fig. 1). Larose et al. (2002) hypothesized that once the AM fungus is in vicinity of the root, fungal

elicitors modulate the flavonoid pattern in the root to improve the conditions for later root colonization.

A different role of flavonoids during root colonization was proposed by Akiyama et al. (2002). In roots of P-deficient melon plants, the flavonoid isovitexin 2''-O- $\beta$ -glucoside was detected, but not in melons with a high P status. Application of the flavonoid to melon plants inoculated with AM fungi enhanced root colonization, demonstrating its stimulatory effect on root colonization by AM fungi.

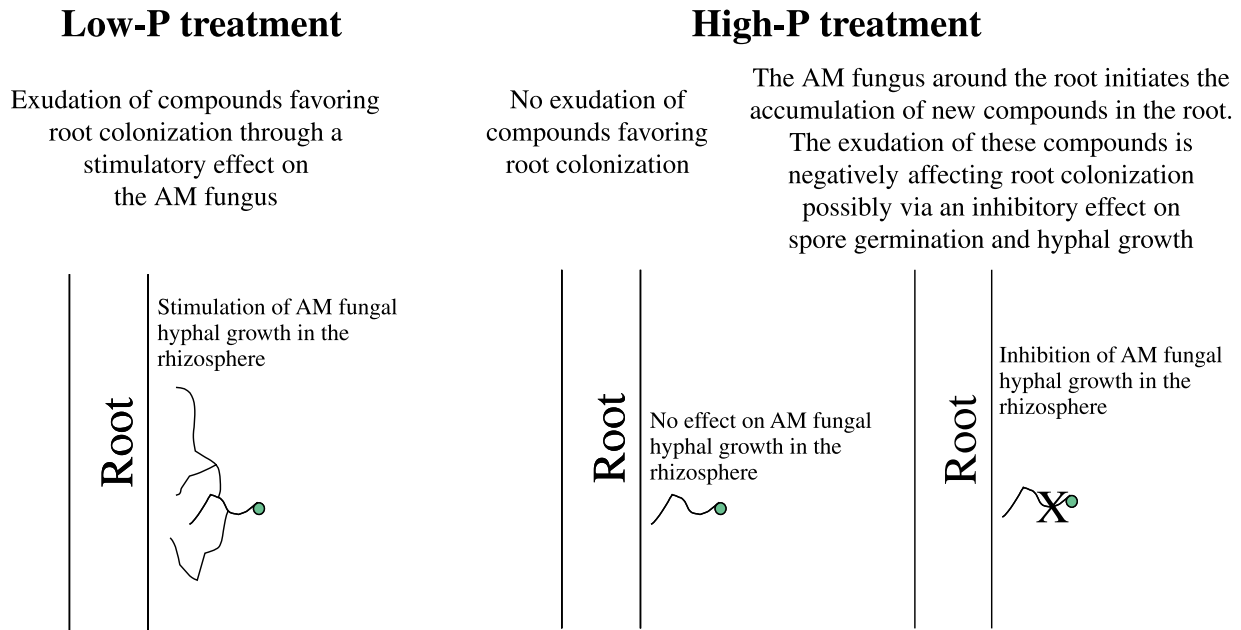
To summarize, depending on the P status, plants possibly have developed different strategies with flavonoids to regulate AM root colonization (Fig. 2). Flavonoid accumulation in roots that is P-status dependent might translate into an altered root exudation. Plants with a high P status, when challenged with signals derived from AM fungi, can accumulate flavonoids inhibitory to AM fungi, thus avoiding the costs of the symbiosis without P nutritional profit, whereas in P-deficient plants, in the absence and presence of signals derived from AM fungi, flavonoids can be accumulated that stimulate root colonization, thus improving the P status of the plant via the P provided by the fungus.

### The AM fungus gets established and the mature AM association

In host-pathogen interactions a range of defense mechanisms is activated in response to a microbial attack. There is some information about defense responses during penetration of AM fungi in the root, the internal growth of the AM fungus in the root, and the development of arbuscules (Harrison 1999; García-Garrido and Ocampo 2002).

In arbuscule-containing cells, members of different classes of plant defense genes, such as genes encoding phenylpropanoid metabolism enzymes (Harrison and Dixon 1993; Volpin et al. 1994, 1995), plant hydrolases (Lambais and Mehdy 1993; Blee and Anderson 2000), and many more (recently reviewed by García-Garrido and Ocampo 2002), have been detected. However, scarce clear data are available

**Fig. 2.** Scheme for a regulation of root colonization by arbuscular mycorrhizal (AM) fungi that is possibly P-status dependent.



**Table 3.** Possible regulatory events in the mature arbuscular mycorrhizal (AM) association.

Event	Reference
Autofluorescing collapsed arbuscules (possibly an indication for the accumulation of phenolics)	Vierheilig et al. 1999, 2001
Accumulation of jasmonic acid (JA)	Hause et al. 2002; Vierheilig and Piché 2002
Accumulation of phytoalexins	Larose et al. 2002; Morandi et al. 1984
Accumulation of H <sub>2</sub> O <sub>2</sub> (oxidative burst)	Salzer et al. 1999
Systematic suppression of further root colonization	Vierheilig et al. 2000a, 2000b; Catford et al. 2003
No accumulation of compounds stimulatory for root colonization	Akiyama et al. 2002
Root exudates lose their stimulatory effect on hyphal growth	Piniór et al. 1999
Root exudates exhibit an inhibitory effect on sporulation and root colonization	J.M. García-Garrido and J.A. Ocampo, personal communication; Piniór et al. 1999; Vierheilig et al. 2003

on the mature AM association, when arbuscules start to collapse (Table 3).

Epifluorescence microscopy (blue excitation 420–490 nm) revealed in roots of mature AM associations autofluorescing spots (Ames et al. 1982; Jabaji-Hare et al. 1984; Klingner et al. 1995), which have been identified as collapsed arbuscules (Vierheilig et al. 1999, 2001). Vierheilig et al. (2001) hypothesized that the autofluorescence can be attributed to a different perception of the collapsing arbuscules by the plant. Whereas the active arbuscule possibly masks its presence in the root cell or blocks a reaction of the plant, and thus in terms of activation of plant defense mechanisms remains unperceived by the plant, fungal cell wall components released by the collapsing, disintegrating arbuscule could elicit plant defense responses, for example, the accumulation of phenolics. Phenolic compounds accumulating in plant cells as a plant defense response against microorganisms are known to fluoresce (Jahnen and Hahlbrock 1988) and thus might be responsible for the autofluorescence of collapsing arbuscules.

A different perception of a viable arbuscule compared

with a collapsed arbuscule in a mature association is in line with observation in several studies. The induction of H<sub>2</sub>O<sub>2</sub> synthesis in plants, the so-called oxidative burst, is a powerful plant defense mechanism against microorganisms (Lamb and Dixon 1997). In root segments of *M. truncatula* colonized by the AM fungus *G. intraradices*, H<sub>2</sub>O<sub>2</sub> accumulated in cells containing collapsed arbuscules, but never in cells with highly branched arbuscules (Salzer et al. 1999).

Phytoalexins, such as glyceollin, are thought to play an important role in the active defense reaction of plants against incompatible pathogens. In mycorrhizal soybeans the accumulation of glyceollin depended on the stage of root colonization. Whereas glyceollin was not accumulated during early stages of root colonization by AM fungi (Wyss et al. 1991), several weeks after inoculation with different AM fungi, in a mature AM association, glyceollin levels had increased (Morandi et al. 1984; Wyss et al. 1991).

Flavonoids play a significant role in how plants interact with organisms in the environment and have been proposed as regulatory compounds in the AM symbiosis (reviewed by Vierheilig et al. 1998a; Morandi 1996). Flavonoid levels

in mycorrhizal plants are modulated by the developmental stage of the AM symbiosis (Larose et al. 2002; Harrison and Dixon 1993). Whereas during root penetration and the establishment of the AM fungus in alfalfa roots intermediate levels of a number of flavonoids are detected, at a late stage of root colonization characterized by viable arbuscules and abundant collapsed arbuscules, high levels of flavonoids such as the phytoalexin medicarpin can be found (Larose et al. 2002).

Jasmonic acid (JA) and its methyl ester have been found in a wide range of plant species. They are known to regulate a number of physiological processes in plants and play a role in interplant signalling and in signal transduction in relation to defense-gene induction (Dong 1998). In mycorrhizal plants JA levels are increased (Hause et al. 2002; Vierheilig and Piché 2002). Following the JA accumulation pattern in mycorrhizal barley plants, Hause et al. (2002) suggested that "...the rise in jasmonates might be related to the fully established symbiosis rather than to the recognition of interacting partners or the onset of interaction".

To localize JA accumulation in situ, hybridization and immunocytochemical analysis of allene oxide synthase, a gene coding for an enzyme involved in JA synthesis and of a jasmonate-induced protein, were performed (Hause et al. 2002). The data clearly revealed that both enzymes are localized in arbuscule-containing cells.

Collapsed arbuscules autofluoresce, but fail to be stained by standard staining techniques, whereas viable arbuscules are stained but do not autofluoresce (Vierheilig et al. 2001). A comparison of autofluorescing arbuscules and stained arbuscules shows clear differences of the number of stained and of autofluorescing arbuscules in roots of host plants (Gange et al. 1999).

Unfortunately, in the work of Hause et al. (2002) arbuscules were not stained with one of the standard staining techniques (Gerdemann 1955; Phillips and Hayman 1970; Brundrett et al. 1984; Koske and Gemma 1989; Nicolson 1959; Vierheilig et al. 1998b; Vierheilig and Piché 1998) to differentiate between labelled collapsed arbuscules and labelled viable arbuscules; thus, it remained unclear whether collapsed arbuscules or viable arbuscules were labelled. Having a closer look at the in situ hybridization of allene oxide synthase showed a labelled structure (Hause et al. 2002) reminiscent of the clumped structures reported as collapsed arbuscules (Vierheilig et al. 2001), indicating that JA synthesis is linked to the presence of collapsed arbuscules.

Looking at the time course of JA accumulation and arbuscule development seems to confirm this hypothesis. About 12 d after inoculation the number of stained arbuscules in barley roots started to drop drastically, and at the same time point the JA accumulation increased sharply (Hause et al. 2002). The life-span of an arbuscule has been estimated at around 7 d (Toth and Miller 1984; Brundrett et al. 1985; Alexander et al. 1988, 1989); thus, JA accumulation seems to start when first arbuscules start to collapse.

We could show that a drop in viable stainable arbuscules in barley roots is followed by an increase of nonstainable collapsed arbuscules (Fig. 3; M. Nell and H. Vierheilig, unpublished data). This could mean that enhanced JA levels in mycorrhizal roots are linked to an increase in the number of collapsed arbuscules.

To summarize, although there are abundant data on the arbuscule as the "nutrition" organ in the AM symbiosis, further studies are needed on the possible role of this organ apart from the nutritional aspect, such as the involvement in the regulation of mycorrhization in the mature association.

## Autoregulation of mycorrhization

Legumes can form a symbiosis not only with AM fungi, but also with nodule-forming, N-fixing rhizobial bacteria. From the plant's perspective, the development of the rhizobial association is a beneficial but also costly process. Thus, legumes developed regulatory mechanisms to control the number of nodules. Already existing nodules systemically inhibit subsequent nodulation in other parts of the root system. This feedback control is termed autoregulation (for details see review by Caetano-Anollés and Gresshoff 1991).

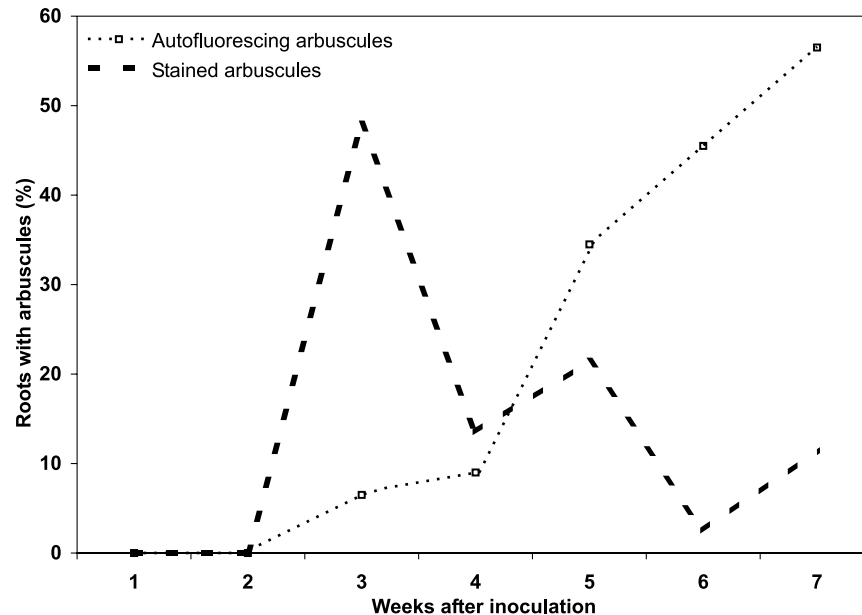
To limit energy expense of the host, a similar mechanism seems plausible in the AM symbiosis. In split-root systems of barley, Vierheilig et al. (2000b) reported that once AM fungi have colonized roots on one half of a split root system, further root colonization by an AM fungus on the other half of the split-root system is reduced. The mechanism is unspecific, meaning that precolonization of one half of a split-root system with *Glomus mosseae*, *G. intraradices*, or *Gigaspora rosea* suppressed subsequent colonization on the other half of the split-root system by *G. mosseae*.

An improved P status and the competition for carbohydrates within the root system of mycorrhizal plants have been suggested as possible factors affecting further root colonization (Pearson et al. 1993). In split-root systems colonized on one side by an AM fungus, the P content was similar to the P content in split-root systems not precolonized by an AM fungus; however, in the precolonized root system further root colonization was suppressed, whereas without precolonization no suppression of root colonization could be observed (Vierheilig et al. 2000b), thus excluding P as a suppressional factor of further root colonization in mycorrhizal plants. Moreover, in a P-application experiment it could be demonstrated that in split-root systems the improved P status and the observed suppressional effect following mycorrhization cannot be linked (Vierheilig et al. 2000c).

Estimated carbon costs of the mycobiont in a mycorrhizal plant are around 4%–20% (Bago et al. 2000; Douds et al. 2000). In a recent study on carbon partitioning in mycorrhizal split-root systems of barley and maple, Lerat et al. (2003a) reported that AM fungi exhibit a fungal-specific sink strength, which is correlated with the degree of colonization. This means that the higher the root colonization by *G. intraradices* and *Gigaspora rosea* was, the more carbon was transferred to the mycorrhizal root. In the split-root system this could mean that once one side of the root system is colonized by AM fungi, less carbon is available on the other side of the split-root system to support root colonization by AM fungi.

Working with *G. mosseae* (BEG 12), Vierheilig (2004) observed that high root colonization on one side of a split-root system results in strong suppression of root colonization on the other side, whereas lower root colonization results in weaker suppression of root colonization on the other side.

**Fig. 3.** Quantification of autofluorescing and stained arbuscules in barley roots in a time-course study. Barley plants were inoculated with the arbuscular mycorrhizal (AM) fungus *Glomus mosseae* (BEG 12) (for details see Vierheilig et al. 2000). In a time-course study, plants were harvested. Starting about 2 cm from the seed, three 1 cm long pieces (meaning root pieces 2, 3, and 4 cm from the seed) from the fresh, living root were collected, mixed, and observed in water with epifluorescence microscopy. The number of roots with autofluorescing spots was quantified (McGonigle et al. 1990). Autofluorescing spots have been reported to be collapsed arbuscules (Vierheilig et al. 1999, 2001). The same root pieces were stained (Vierheilig et al. 1998b), and after staining the number of roots with stained arbuscules was quantified (McGonigle et al. 1990; M. Nell and H. Vierheilig, unpublished data).



These results seem to confirm carbon partitioning as an explanation for suppression of further root colonization in mycorrhizal plants. Interestingly, when looking at carbon partitioning in barley plants colonized by the *G. mosseae* BEG 12 isolate, the fungus showed no carbon-sink strength independently of the degree of root colonization (Lerat et al. 2003a). Carbon-partitioning experiments with other *G. mosseae* isolates showed that the lack of sink strength of the isolate BEG 12 is not a general characteristic of *G. mosseae* (Lerat et al. 2003b).

As AM fungi with high carbon-sink strengths exhibit a similar suppressional effect on further root colonization as AM fungi without any carbon-sink strength, it can be excluded that the observed reduction of root colonization in split-root systems precolonized by AM fungi is due to a competition for carbohydrates between an already established AM fungus and a latercolonizing AM fungus. The role of carbon as a limiting factor for AM fungal root colonization has been questioned before (Vierheilig et al. 2002), and it has been suggested that carbon might play a role in the spreading of the AM fungus in the root after the fungus has penetrated the root and formed its exchange organ, the arbuscules, but not before.

Vierheilig (2004) reported that in a split-root system precolonized on one side with an AM fungus, the suppressional effect on AM fungal root colonization increases with the degree of root colonization. The degree of root colonization was linked with the period when plants were inoculated. Barley plants that were inoculated earlier showed a higher degree of root colonization than plants that were inoculated later.

The time course of the appearance of autofluorescing collapsed arbuscules in roots of barley plants inoculated with AM fungi has been discussed previously (see “The AM fungus gets established and the mature AM association” and Fig. 3). As an autoregulatory effect in mycorrhizal plants is only observed at high colonization levels, which means at a later stage of the symbiosis (Vierheilig 2004), further root colonization of a mycorrhizal plant might only be suppressed when arbuscules start to collapse and thus activate defense responses of the plant, thus reducing further root colonization by AM fungi.

### Autoregulation of mycorrhization and nodulation

Growing evidence is brought forward that a number of processes in the rhizobial and mycorrhizal association are similarly regulated (reviewed by Hirsch and Kapulnik 1998; Albrecht et al. 1999; Guinel and Geil 2002; Stracke et al. 2002; Staehelin et al. 2001; Vierheilig and Piché 2002). Autoregulation of mycorrhization and nodulation also seems to share some common features. Autoregulation of nodulation occurs only after a critical number of nodules is formed on the root system (see review by Caetano-Anollés and Gresshoff 1991). Similarly in split-root systems of barley, a clear suppressional effect on further root colonization in mycorrhizal barley plants was only observed when one side of the split-root system was heavily mycorrhizal, but not at lower colonization levels (Vierheilig 2004).

Recent results show other interesting parallels in the autoregulation of both symbioses. Catford and coworkers (2003)

reported that in alfalfa plants an established mycorrhizal association systemically suppresses not only further mycorrhization but also nodulation. Vice versa, nodulation on one side of a split-root system systemically suppresses later mycorrhization on the other side of the split-root system. These results point towards a similar regulatory mechanism in the rhizobial and the AM association suppressing either nodulation or mycorrhization.

van Brussel et al. (2002) reported that application of Nod factors from *Rhizobium leguminosarum* bv. *viciae* to one side of a split-root system inhibited nodule formation of *Vicia* plants on the other side. Their observations indicate that nodulation is not a prerequisite for suppression of further nodule formation but that Nod factors alone can elicit an autoregulatory feedback response. Recent experiments show a similar effect of Nod factors in the interaction between alfalfa and *Sinorhizobium meliloti* (Catford et al. 2003). Moreover, we found that application of Nod factors to one side of a split-root system reduced AM fungal colonization on the other side of the split-root system. Carbon partitioning in split-root systems of alfalfa plants was unaffected by Nod factor application, excluding a carbon-limitation effect on nodulation and mycorrhization (Catford et al. 2003). The data indicate that Nod factors trigger a secondary signal involved in autoregulation of nodulation, which initiates blockage of AM fungal colonization. In this context, it is worth mentioning that Nod factors may act as chitoooligosaccharide elicitors on legumes and nonlegumes (Staehelin et al. 1994; Müller et al. 2000), thereby inducing plant defense reactions (Savouré et al. 1997; Xie et al. 1999).

## The root exudation of mycorrhizal plants

There is abundant information on the stimulatory effect of root exudates from nonmycorrhizal plants on different stages during the development of the AM symbiosis (e.g., Graham 1982; Elias and Safir 1987; Bécard and Piché 1989; Gianinazzi-Pearson et al. 1989; Tawaraya et al. 1996, 1998; reviewed by Vierheilig et al. 1998a). Recently, root exudates of tomato mutants resistant to AM fungal colonization (Myc<sup>-</sup> phenotype) showed an inhibitory effect on spore germination and hyphal growth, demonstrating that root exudates can be important factors in the control of colonization of host plants by AM fungi (David-Schwartz et al. 2003; Gadkar et al. 2003).

The mechanisms regulating the mycorrhizal auto-regulation are still unclear; recently, however, Vierheilig and Piché (2002) suggested that an altered root exudation might be at least partially responsible for the altered susceptibility of mycorrhizal plants to further root colonization by AM fungi.

Around mycorrhizal roots the microbial population is modified (Bansal and Mukerji 1994; Linderman and Paulitz 1990; Andrade et al. 1997; Filion et al. 1999; Vazquez et al. 2000; Marschner et al. 2001). These changes have been attributed to an altered root exudation of mycorrhizal plants. There are some reports on changes in root exudates of mycorrhizal plants. Higher concentrations of phenolics were found in mycorrhizal potato root exudates compared with

exudates from nonmycorrhizal roots (McArthur and Knowles 1992), whereas other nonidentified compounds were only detected in nonmycorrhizal P-deficient parsley but were absent in root exudates of mycorrhizal plants (Franken and Gnädinger 1994).

Root exudates of mycorrhizal plants have been reported to exhibit a different effect on AM fungi than root exudates from nonmycorrhizal roots. Root exudates of nonmycorrhizal plants stimulate hyphal growth (e.g., Graham 1982; Elias and Safir 1987; Bécard and Piché 1989; Gianinazzi-Pearson et al. 1989; Tawaraya et al. 1996); however, no stimulatory effect on hyphal growth could be detected when root exudates of mycorrhizal plants were tested (Pinior et al. 1999).

Using an experimental system to study the effect of root exudates on the development of the AM symbiosis, Tawaraya et al. (1998) reported that root exudates of P-deficient, nonmycorrhizal plants stimulate AM root colonization; however, when root exudates of mycorrhizal plants were tested in the same experimental setup, an inhibitory effect on root colonization was observed (Pinior et al. 1999). Recently, using modified two-compartment Petri dishes, with one compartment containing Ri-T DNA-transformed carrot roots colonized by *G. intraradices* (for details of the nonmodified system, see St-Arnaud et al. 1996), which allow the exchange of exudates between compartments and hyphal growth in both compartments, but restrict root growth to one compartment, it was reported that in presence of diffusible exudates of the mycorrhizal Ri-T DNA-transformed carrot roots, sporulation of the AM fungus in the root-free compartment is reduced, and the presence of a factor in the root exudates negatively affecting AM fungi was suggested (J.M. García-Garrido and J.A. Ocampo, personal communication).

Alterations of the exudation pattern seem not to be limited to mycorrhizal roots, but, through a plant-mediated mechanism, also occur in nonmycorrhizal roots of a mycorrhizal plant. When Vierheilig et al. (2003) tested the effect of root exudates obtained from a split-root system of cucumber (one side mycorrhizal and the other side nonmycorrhizal) on root colonization, the fact that exudates from both sides exhibited a similar inhibitory effect shows that changes of the pattern in mycorrhizal plants are systemic, meaning that the changes are not limited to the mycorrhizal root.

Systemic changes of the exudation pattern of mycorrhizal plants have been confirmed with other soilborne microbial populations. When comparing the bacterial community structure around mycorrhizal and nonmycorrhizal roots of a split-root system of maize, Marschner and Baumann (2003) found that changes of the bacterial community structure were similar on the mycorrhizal and the nonmycorrhizal side, differing on both sides from the bacterial community structure around roots of nonmycorrhizal plants.

All these data indicate alterations of the root exudation pattern in mycorrhizal plants, which have an effect on the microbial population, including AM fungi around mycorrhizal roots. An analysis of root exudates from mycorrhizal plants and nonmycorrhizal plants to identify the compounds involved in the differing effect of these exudates seems most interesting.

## Perspectives

While the existence of a “Myc factor” is an exciting hypothesis that needs further studies to elucidate the character of the involved signal and its significance for the establishment of the symbiosis, the mechanism regulating further root colonization by AM fungi in already mycorrhizal plants could be interesting in another context.

There are abundant data on a bioprotective effect of mycorrhizal plants on soilborne fungal pathogens (Dehne 1982). The mechanisms involved are still a matter of debate (Azcón-Aguilar et al. 2002); however, it is generally accepted that a well-established AM symbiosis, characterized by the formation of arbuscules, is a prerequisite for a mycorrhizal-induced increase in resistance (Caron et al. 1986; Cordier et al. 1998; Slezacek et al. 2000). This reminds us of the autoregulatory mechanism of mycorrhization discussed previously, also depending on a well-established AM symbiosis (Vierheilig 2004).

Moreover, looking at the alterations in the root exudation of a mycorrhizal plant, it is tempting to speculate that not only the susceptibility of the plant to AM fungi but also to other soilborne fungi is affected (see Vierheilig and Piché 2002). This seems confirmed in a study by Norman and Hooker (2000) reporting that the sporulation of the fungal pathogen *Phytophthora fragariae* shows greater stimulation by exudates of nonmycorrhizal strawberry plants than by mycorrhizal strawberry plants.

These data are in line with the hypothesis of Vierheilig and Piché (2002) suggesting that plants colonized by AM fungi while trying to limit their costs of the AM symbiosis also acquired bioprotection against pathogenic fungi. It seems plausible that an already mycorrhizal plant develops only one mechanism to repulse colonization by fungi, not discriminating between AM fungi and soilborne pathogenic fungi. Although the presented data do point towards “one mechanism, two symptoms” for autoregulation and bioprotection in mycorrhizal plants, further studies are needed to test the validity of the presented hypothesis.

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