

REVIEW

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Arbuscular mycorrhizas and biological control of soil-borne plant pathogens – an overview of the mechanisms involved

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Abstract Biological control of plant pathogens is currently accepted as a key practice in sustainable agriculture because it is based on the management of a natural resource, i.e. certain rhizosphere organisms, common components of ecosystems, known to develop antagonistic activities against harmful organisms (bacteria, fungi, nematodes etc.). Arbuscular mycorrhizal (AM) associations have been shown to reduce damage caused by soil-borne plant pathogens. Although few AM isolates have been tested in this regard, some appear to be more effective than others. Furthermore, the degree of protection varies with the pathogen involved and can be modified by soil and other environmental conditions. This prophylactic ability of AM fungi could be exploited in cooperation with other rhizospheric microbial antagonists to improve plant growth and health. Despite past achievements on the application of AM in plant protection, further research is needed for a better understanding of both the ecophysiological parameters contributing to effectiveness and of the mechanisms involved. Although the improvement of plant nutrition, compensation for pathogen damage, and competition for photosynthates or colonization/infection sites have been claimed to play a protective role in the AM symbiosis, information is scarce, fragmentary or even controversial, particularly concerning other mechanisms. Such mechanisms include (a) anatomical or morphological AM-induced changes in the root system, (b) microbial changes in rhizosphere populations of AM plants, and (c) local elicitation of plant defence mechanisms by AM fungi. Although compounds typically involved in plant defence reactions are elicited by AM only in low amounts, they could act locally or transiently by making the root more prone to react against pathogens. Current research based on molecular, immunological

and histochemical techniques is providing new insights into these mechanisms.

Key words Antagonistic microorganisms · Arbuscular mycorrhizas · Biocontrol · Plant-defence response · Sustainability

Introduction

A key, universally accepted concept is that natural microbial populations in soil or other “living” substrate are activated to grow around developing plant roots, giving rise to the so-called “rhizosphere” (Azcón-Aguilar and Barea 1992). As a rhizosphere develops at the root-soil interface, microorganisms there interact with both plant roots and soil constituents. Communication among the different components is mainly via chemical or biochemical signals, although physical interaction is also possible. This “dialogue” is modulated by inherent soil characteristics and the prevailing environmental conditions of the ecosystem (Bowen 1980; Lynch 1990).

The most important interactions developing in the rhizosphere can be classified into three main groups: (1) plant – plant interactions caused by overlapping rhizospheres, which results in competition for nutrients; (2) root–microorganism interactions, determined by plant activities that stimulate microorganisms to grow around the roots (rhizosphere effect) and by microbial activities that affect plant development, either by benefiting the plant or by inducing disease; and (3) microbe–microbe interactions, which include both synergistic and antagonistic activities (Stotzky 1972; Lynch 1990).

Biological control can be defined as the directed, accurate management of common components of ecosystems to protect plants against pathogens. In this regard, microbial diversity is a key natural resource (Kennedy and Smith 1995). Thus, biological control preserves environmental quality by a reduction in chemical inputs,

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and is characteristic of sustainable management practices (Altieri 1994; Barea and Jeffries 1995).

Under natural conditions, plants strictly speaking do not have roots, they have mycorrhizas; the roots of most flowering plants form mutualistic symbioses with certain soil fungi (Harley and Smith 1983). Mycorrhizal associations are found in nearly all ecological situations, with arbuscular mycorrhizas (AM) being the most common type in normal cropping systems and in natural ecosystems (Harley and Smith 1983; Gianinazzi and Schüepp 1994). AM fungi, which belong to the order Glomales of the Zygomycetes (Rosendahl et al. 1994), biotrophically colonize the root cortex and develop an extramatrical mycelium which helps the plant acquire mineral nutrients and water from the soil. AM symbioses play a key role in nutrient cycling in ecosystems (Jeffries and Barea 1994), and the external mycorrhizal mycelium, in association with other soil organisms, forms water-stable aggregates necessary for good soil quality (Bethlenfalvy and Schüepp 1994).

It is evident that an increased capacity for nutrient acquisition resulting from mycorrhiza association could help the resulting stronger plants to resist stress. However, AM symbioses may also improve plant health through a more specific increase in protection (improved resistance and/or tolerance against biotic and abiotic stresses) (Bethlenfalvy and Linderman 1992; Barea and Jeffries 1995).

The study of a possible role for AM symbiosis in protection against plant pathogens began in the 1970s, and a great deal of information has been published on the subject, however, we still know very little about the underlying mechanisms (Hooker et al. 1994; Linderman 1994). It is not our aim here to discuss all the information in the many published papers. Instead, we will analyse the mechanisms by which AM fungi could control root pathogens and indicate possible fruitful research approaches.

Arbuscular mycorrhizas as biocontrol agents

Detailed review articles summarizing and discussing results on AM and biological control include those by Schönbeck (1979), Dehne (1982), Bagyaraj (1984), Schenck (1987), Smith (1987), Zambolin (1987), Caron (1989), Jalali and Jalali (1991), Paulitz and Linderman (1991), Sharma et al. (1992), Hooker et al. (1994) and Linderman (1994). The main conclusions that can be drawn are: (1) AM associations can reduce damage caused by soil-borne plant pathogens, (2) the abilities of the AM symbioses to enhance resistance or tolerance in roots are not equal for the different AM fungi so far tested, (3) protection is not effective for all pathogens, and (4) protection is modulated by soil and other environmental conditions. Thus it can be expected that interactions between different AM fungi and plant pathogens will vary with the host plant and the culture system.

Consistent reduction of disease symptoms has been described for fungal pathogens such as *Phytophthora*, *Gaeumannomyces*, *Fusarium*, *Chalara* (*Thielaviopsis*), *Pythium*, *Rhizoctonia*, *Sclerotium*, *Verticillium*, *Aphanomyces*, and for nematodes such as *Rotylenchus*, *Pratylenchus* and *Meloidogyne*. This does not mean that AM formation will be effective against these pathogens under all circumstances. In fact, an AM-induced increase in resistance or a decrease in susceptibility requires the pre-establishment of AM and extensive development of the symbiosis before pathogen attack. Furthermore, the potential effectiveness of a biological control agent depends on the virulence and inoculum potential of the pathogen(s) present in the soil. A high pathogen inoculum density in the rhizosphere may render ineffective any form of biocontrol, including that mediated by an AM symbiosis.

It is also evident from several studies that the prophylactic property of AM fungi can be exploited in association with other rhizosphere microbial antagonists. In this respect, there is increasing interest in plant-growth-promoting rhizobacteria such as *Pseudomonas* and *Bacillus*, in bacteria with rhizogenetic effects such as *Agrobacterium*, and in fungal agents of biological control such as *Trichoderma* and *Gliocladium*.

The protective effects of AM inoculation may be both systemic and localized, and there is evidence supporting both types of induced resistance (Linderman 1994).

Mechanisms by which an AM association could control root pathogens

Mechanisms that could account for the protective activity ascribed to AM fungi include improvement of plant nutrition, root damage compensation, competition for photosynthates or colonization/infection sites, production of anatomical or morphological changes in the root system, changes in mycorrhizosphere microbial populations, and activation of plant defence mechanisms.

Improved nutrient status of the host plant

Since increased nutrient uptake made possible by the AM symbiosis results in more vigorous plants, the plant itself may thus be more resistant to or tolerant of pathogen attack. Although in many studies of improved nutrition as a mechanism for disease control, enhanced P nutrition could account for the higher tolerance of mycorrhizal plants to pathogens (either fungus or nematode), there are a number of contradictory reports (Hooker et al. 1994; Linderman 1994). For example, P-tolerant AM fungi reduced nematode effects even under high-P conditions, indicating that non-P-mediated mechanisms are involved, probably physiological changes in the roots (Smith 1987; Zambolin 1987).

Damage compensation

It has been suggested that AM fungi increase host tolerance of pathogen attack by compensating for the loss of root biomass or function caused by pathogens (Linderman 1994), including nematodes (Pinochet et al. 1996) and fungi (Cordier et al. 1996). This represents an indirect contribution to biocontrol through the conservation of root-system function, both by fungal hyphae growing out into the soil and increasing the absorbing surface of the roots and by the maintenance of root cell activity through arbuscule formation (Cordier et al. 1996).

Competition for host photosynthates

It has been proposed that the growth of both the AM fungi and root pathogens depends on host photosynthates and that they compete for the carbon compounds reaching the root (Smith 1987; Linderman 1994). When AM fungi have primary access to photosynthates, the higher carbon demand may inhibit pathogen growth. However, there is little or no evidence that competition for carbon compounds is a generalized mechanism for pathogen biocontrol activity of AM symbiosis.

Competition for infection/colonization sites

Pioneering observations (see Dehne 1982) illustrated how fungal root pathogens and AM fungi, although colonizing the same host tissues, usually develop in different root cortical cells, indicating some sort of competition for space.

Both localized and nonlocalized mechanisms could exist, probably depending on the pathogen (fungus, nematode). Reports by Jalali and Jalali (1991) and Linderman (1994) point to a localized effect, whilst the reviews by Dehne (1982) and Smith (1987) suggest that the extent of the protection can not be explained by a localized mechanism alone. Cordier et al. (1996) showed that *Phytophthora* development is reduced in AM fungal-colonized and adjacent uncolonized regions of AM root systems, and that in the former the pathogen does not penetrate arbuscule-containing cells. This means that localized competition occurs, and that even in the absence of systemic resistance, resistance was still induced at some distance from the AM-colonized tissue.

Anatomical and morphological changes in the root system

It has been demonstrated that AM colonization induces remarkable changes in root system morphology, as well as in the meristematic and nuclear activities of root

cells (Atkinson et al. 1994). This might affect rhizosphere interactions and particularly pathogen-infection development. The most frequent consequence of AM colonization is an increase in branching, resulting in a relatively larger proportion of higher order roots in the root system (Hooker et al. 1994). However, the significance of this finding for plant protection has not yet been sufficiently considered. In most studies on AM fungi and biocontrol, the roots have not been examined for anatomical changes. Thus, more attention needs to be given to root system morphology in the future because it could modify the infection dynamics of the pathogen as well as the pattern of resistance of AM roots to pathogen attack.

Microbial changes in the mycorrhizosphere

AM formation induces changes in host physiology that can be decisive for root exudation patterns (Azcón-Aguilar and Bago 1994; Smith et al. 1994) and, consequently, cause qualitative and/or quantitative alterations in microbial populations in the rhizosphere. There is evidence that microbial shifts occur in the mycorrhizosphere and that the resulting microbial equilibria could influence the growth and health of plants. Although this effect has not been specifically evaluated as a mechanism for AM-associated biological control, there are indications that such a mechanism does operate (Azcón-Aguilar and Barea 1992; Linderman 1994; Barea et al. 1996).

Changes in soil microorganism populations induced by AM formation may lead to stimulation of certain components of the microbiota, which in turn may be antagonistic to root pathogens. Meyer and Linderman (1986) showed that AM establishment can change both the total population and specific functional groups of microorganisms in the rhizoplane or the rhizosphere soil. Further studies have corroborated these findings and demonstrated that such an effect is dependent on the AM fungus involved (Linderman 1994). Meyer and Linderman (1986) found that the number of sporangia and zoospores formed by cultures of *Phytophthora cinnamomi* was reduced by the application of extracts of rhizosphere soil from AM plants. Secilia and Bagyaraj (1987) isolated more pathogen-antagonistic actinomycetes from the rhizosphere of AM plants than from nonmycorrhizal controls, an effect that also depended on the AM fungus involved. Furthermore, Caron (1989) reported a reduction in *Fusarium* populations in the soil surrounding mycorrhizal tomato roots as compared with the soil of nonmycorrhizal controls. These studies merit further attention in order to elucidate how microbiota-mediated changes may be involved in biological control by AM associations.

The prophylactic ability of some AM fungi could be exploited in association with other rhizosphere microorganisms known to be antagonistic to root pathogens that are being used as biological control agents (Lin-

derman 1994; Barea et al. 1996). As previously mentioned, among the microorganisms known to be antagonists of fungal pathogens are fungi such as *Trichoderma* and *Gliocladium* and rhizobacteria such as *Pseudomonas* and *Bacillus* (Kloepper et al. 1991; Linderman 1994). Rhizobacteria aggressively colonize the root-soil interface, where they establish and maintain a large number of cells. Although some of these bacteria can be deleterious, others (PGPR) are able to promote plant growth by several mechanisms (Kloepper et al. 1991; Linderman 1992, 1994; Glick 1995; Leeman et al. 1995). The ecological significance of PGPR is being intensively studied because of their role in biological control of plant pathogens (Kloepper 1992; Linderman 1994). Their effects are mainly due to the capacity of PGPR to colonize the rhizosphere soil and the rhizoplane and to diminish populations of deleterious organisms. They do this mainly by the production of antibiotics, hormones, siderophores and HCN, and by competition for colonization sites and carbon compounds (Kloepper 1992; Leeman et al. 1995, 1996). The molecular bases of the biocontrol by rhizobacteria are now being investigated (O'Gara et al. 1994; Cook et al. 1995). Systemic-induced resistance has been proposed to be a mechanism of disease suppression by PGPR (Leeman et al. 1995, 1996).

Apart from their effect on plant performance through indirect biological control, some PGPR can directly promote root and shoot growth, nodule formation by *Rhizobium* (Nodulation Promoting Rhizobacteria, NPR), seedling emergence (EPR) (Kloepper et al. 1991) and, in some cases, mycorrhiza establishment (MPR or, as they are usually known, mycorrhiza-helper-bacteria MHB) (Garbaye 1994; Barea et al. 1996).

AM fungi and PGPR may cooperate in several ways, including their mutual establishment in the rhizosphere, improvement in plant rooting, enhancement of plant growth and nutrition, biological control of root pathogens, and improved nodulation in the case of legumes (Barea et al. 1996).

Preliminary studies suggest that microbial antagonists of fungal pathogens, either fungi or PGPR, do not antagonize AM fungi. Moreover, they can improve the development of the mycosymbiont and facilitate AM formation (see Linderman 1994; Barea et al. 1996). This has been shown particularly for *Trichoderma* spp. (Calvet et al. 1993) and for *Pseudomonas* spp. producing 2, 4-diacetylphloroglucinol (Vidal et al. 1996). Therefore, the management of these interactions improving plant growth and health, in an integrated approach, should be one of the main objectives of sustainable agriculture (Bethlenfalvay and Linderman 1992; Barea and Jeffries 1995). Current interest in this topic has led to research on the manipulation of soil microorganisms, particularly with regard to improving the production, formulation and practical use of efficient microbial inoculants (Elliot and Lynch 1995). Recent developments in molecular biology techniques and the application of novel biotechnological approaches are

facilitating a more accurate exploration of the natural diversity of soil microorganisms for the isolation of new strains and the generation of genetically-modified superior rhizobacteria strains. Improved microbial inoculants may become available as biofertilizers or for the biocontrol of plant disease (O'Gara et al. 1994). However, the existing barriers to growing AM fungi in pure culture are still holding back a parallel AM inoculum development and application.

Activation of plant defence mechanisms

It is likely that AM associations as agents in biological control will be acting by more than one mechanism. The activation of specific plant defence mechanisms as a response to AM colonization is an obvious basis for the protective capacity of AM fungi. The elicitation by an AM symbiosis of specific plant defence reactions could predispose the plant to an early response to attack by a root pathogen (Gianinazzi-Pearson et al. 1994). However, the general conclusion from the research carried out since the review by Koide and Schreiner (1992) is that only a weak or very local, transient activation of plant defence mechanisms occurs during AM formation (Gianinazzi-Pearson et al. 1994, 1996). Current research using molecular biology techniques and immunological and histochemical analyses will probably provide more information about these mechanisms. This methodology may detect substances and/or reactions elicited only to low levels by AM formation but still in some way involved in plant protection. From here on this review will focus on the analysis of plant defence responses and on their possible triggering by the establishment of an AM symbiosis.

During their life cycle plants evolve a number of defence responses elicited by various signals, including those associated with pathogen attack (Huynh et al. 1992). Among the compounds involved in plant defence (Bowles 1990) studied in relationship to AM formation are phytoalexins, enzymes of the phenylpropanoid pathway, chitinases, β -1,3-glucanases, peroxidases, pathogenesis-related (PR) proteins, callose, hydroxyproline-rich glycoproteins (HRGP) and phenolics (Gianinazzi-Pearson et al. 1994).

Electrophoretic analysis of soluble extracts from AM roots has demonstrated that the host plant produces a number of new proteins (endomycorrhizins) in response to AM colonization (Gianinazzi-Pearson and Gianinazzi 1995). New polypeptides are synthesized during AM infection (García-Garrido et al. 1993; Dumas-Gaudot et al. 1994) and others disappear (Dumas-Gaudot et al. 1994). However, this altered pattern of protein synthesis in the plant is not necessarily related to defence reactions. This is a research area deserving further attention.

Phytoalexins, low-molecular-weight, toxic compounds usually accumulating with pathogen attack and released at the sites of infection, are not detected dur-

ing the first stages of AM formation but can be found at later stages of the symbiosis (Morandi et al. 1984). The main phytoalexin of soybean glyceollin could not be detected during the first 30 days after AM inoculation, but there was an evident increase in the compound in roots infected by *Rhizoctonia solani* (Wyss et al. 1991). The level of the phytoalexin medicarpin increased transiently during the early stages of AM colonization in *Medicago truncatula*, but decreased to very low levels during later stages of symbiosis development (Harrison and Dixon 1993).

There seems to be a similar low activation of the phenylpropanoid-related enzymes. In particular, both phenylalanine ammonium-lyase (PAL), the first enzyme of the phenylpropanoid pathway, and chalcone isomerase, the second enzyme specific for flavonoid/isoflavonoid biosynthesis, increased in amount and activity during early colonization of plant roots by *Glomus intraradices*, but then decreased sharply to levels at or below those in uninoculated controls (Lambais and Mehdy 1993; Volpin et al. 1994, 1995). These results suggest that AM fungi initiate a host defence response which is subsequently suppressed. In contrast, levels of transcripts encoding PAL and chalcone synthase, also involved in the flavonoid/isoflavonoid biosynthesis, increased in *Medicago truncatula* roots during colonization with *Glomus versiforme* (Harrison and Dixon 1993). In a recent study, there was little change in the accumulation of defence-related transcripts in *Glomus intraradices*-colonized roots in comparison with uninoculated controls, when extracts from the entire root system were examined. However, in samples of the same colonized roots subjected to *in situ* hybridization, PAL transcript accumulation was detected in cells containing arbuscules (Blee and Anderson 1996).

Chitinases are little or only transiently induced by AM colonization. Dumas-Gaudot et al. (1992a,b) found new chitinase isoforms that were specifically induced in several AM associations. The molecular weights of these isoforms and variation in their characteristics with the plant but not the fungal species involved point to a plant origin for these new isoenzymes. These chitinase isoforms differ from those elicited by root fungal pathogens (Dumas-Gaudot et al. 1992a), indicating a different pattern of plant response to pathogenic and mutualistic fungi.

Although it has been reported that total chitinase activity is higher in AM roots than in non-mycorrhizal controls (Lambais and Mehdy 1993), it appears that increased levels of chitinase activity are only detected in AM roots at the beginning of colonization (Spanu et al. 1989; Bonfante and Spanu 1992; Lambais and Mehdy 1993). At later stages, enhanced chitinase activity was not observed. A decrease in β -1,3-endoglucanase activity has also been reported at specific stages during mycorrhiza development (Lambais and Mehdy 1993). The decreases were accompanied by differential reductions in the levels of mRNAs encoding for different endochitinase and endoglucanase isoforms. These observations

suggest a systemic suppression of the defence reaction when the AM symbiotic interactions begin to function. However, mRNAs encoding chitinases (Blee and Anderson 1996) and a β -1,3-endoglucanase (Lambais and Mehdy 1995) have been reported to accumulate in and around cells containing arbuscules. This suggests localized induction of specific defence-related genes, which might be involved in the regulation of AM development by controlling intraradical fungal colonization.

Wall-bound peroxidase activity has been detected during initial stages of AM colonization, which later decreases (Spanu and Bonfante-Fasolo 1988). However, peroxidase activity associated with epidermal and hypodermal cells increased in mycorrhizal roots (Gianinazzi and Gianinazzi-Pearson 1992), a process that can contribute to higher resistance to certain root pathogens. Peroxidase activity was not detectable in cells containing intracellular arbuscular hyphae (Spanu and Bonfante-Fasolo 1988). Thus, in contrast to pathogen infection, peroxidases do not appear to be associated with plant control of AM fungal development in the root cortex, or to be linked to arbuscule senescence and death within host cells.

PR proteins and HRGP are synthesized only locally and in very low amounts. In particular, the PR-b₁ proteins are only synthesized in cells containing living arbuscules (Gianinazzi-Pearson et al. 1992). The strong accumulation of HRGP in infections by pathogenic fungi contrasts with the weak reaction observed during AM colonization, although these molecules were regularly distributed around the arbuscular hyphae (Balestrini et al. 1994).

In spite of the fact that phenolic compounds have not been detected in significant amounts, the increased lignification of root endodermal cells induced by AM colonization has been suggested (see Dehne 1982). This deserves further investigation as the process would make penetration of the pathogens into root tissues more difficult.

In summary, only weak responses to AM infection have been observed regarding some activities like lignification, production of phytoalexins and peroxidases and the expression of genes coding for PR proteins, indicating that AM fungi do not elicit typical defence responses. However, these compounds could sensitize the root to pathogens and enhance mechanisms of defence to subsequent pathogen infection; the results of Benhamou et al. (1994) support this hypothesis. These authors compared the responses of AM and non-mycorrhizal transformed carrot roots to infection by *Fusarium oxysporum* f. sp. *chrysanthemi*. In mycorrhizal roots, the growth of the pathogen was usually restricted to the epidermis and cortical tissues, whereas in non-mycorrhizal roots the pathogen reached a higher development, infecting even the vascular stele. The *Fusarium* hyphae inside mycorrhizal roots exhibited a high level of structural disorganization, probably induced by a strong reaction of the host cells characterized by the massive accumulation of phenolic-like compounds and the pro-

duction of hydrolytic enzymes such as chitinases. This strong reaction was not induced by nonmycorrhizal roots, suggesting that the activation of plant defence responses by mycorrhiza formation provides a certain protection against the pathogen. These results, although they need to be confirmed using entire plants, clearly show that AM infection makes the root more responsive to pathogen attack, promoting a quicker and stronger reaction against the pathogen.

In contrast to the weak defence response towards AM fungi found in AM hosts, it is noteworthy that in *myc⁻* pea mutants, AM fungi trigger a strong resistance reaction. This suggests that the AM fungi are able to elicit a defence response, but that symbiosis-specific genes somehow control the expression of the genes related to plant defence during AM establishment (Gianinazzi-Pearson et al. 1994, 1995, 1996). It is curious, in this context, that the constitutive expression of several PRs in tobacco plants did not affect either the time course or the final level of colonization by *Glomus mosseae*, which was only reduced in plants constitutively expressing an acidic isoform of tobacco PR-2, a glucanase (Vierheilig et al. 1996).

Potential for applying AM technology to plant protection against root pathogens

Although it is difficult to reach practical conclusions because of the complexity of the microbe-soil-plant system and the decisive influence of prevailing environmental conditions, it may nevertheless be possible to find the right combination of factors to exploit the prophylactic ability of AM fungi. So far, examples of successful practical application are scarce (Hooker et al. 1994; Linderman 1994). Thus, although further research is needed, existing knowledge suggests that management recommendations for the biological control of target diseases in sustainable agrosystems, particularly with nursery and horticultural crops, could be made in the future. Appropriate AM fungi must be used, preferably in association with other pathogen-antagonistic members of soil microbiota. Additionally, the possible role of AM in biological control must be considered and exploited in plant breeding programmes aimed at selecting pathogen-resistant cultivars.

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