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Interrelationships between vesicular-arbuscular mycorrhiza and rhizosphere microflora in apple replant disease

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Abstract

Phytotoxic micromycetes appear to be responsible for the apple replant disease (ARD). This was suppressed by the inoculation of apple-tree seedlings with some species of vesicular-arbuscular mycorrhizal (VAM) fungi - *Glomus fasciculatum* and *G. macrocarpum*. After the inoculation, growth of apple-tree seedlings improved in dependence on the type of soil, on VAM fungus species and on the ARD appearance. After 12-month cultivation, plant biomass (height, shoot and root dry masses) was markedly increased by inoculation with *G. fasciculatum*. Similarly, the numbers of colony forming units per unit soil (CFU) of phytotoxic micromycetes and of diazotroph bacteria (associative dinitrogen-fixing bacteria) in the rhizosphere was affected; CFU of phytotoxic micromycetes decreased, whereas CFU of the genus *Azospirillum* was higher. These bacteria could also serve as antagonists against phytotoxic micromycetes. It is also suggested that the ratio of CFU of diazotroph bacteria to CFU of phytotoxic micromycetes can be used as an indicator of the degree of ARD. It may be assumed that the use of some VAM fungi can replace the chemical treatment of the soil with ARD.

Introduction

There is no doubt about the significance of VAM fungi in the nutrition and growth of host plants. Many reports indicate the interaction between VAM fungi and plant pathogens (Schenck and Kellam 1978, Schoenbeck 1980, Schoenbeck and Dehne 1981). Mycorrhizal plants are generally more resistant against soil-borne pathogens than non-mycorrhizal plants (Dehne 1981). In dependence on the host plant, on plant nutritional requirements, on the inoculum level of VAM fungi and on the virulence of pathogens mycorrhizal plants suffer less damage and incidence of the disease (Baltruschat 1975, Schoenbeck 1980, Schoenbeck and Dehne 1981, Dehne 1982). Mycorrhizal fungi could be more successful in competition with plant pathogens

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Abbreviations: ARD - apple replant disease; CFU - colony forming units per unit soil [10^4 g^{-1}(soil)]; VAM - vesicular-arbuscular mycorrhiza; WHC - water holding capacity [%].

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for the same host plants in colonization of their roots as a result of induced resistance to parasitic infection.

VAM fungi can increase not only the resistance of plants to soil-borne pathogens, but also to unfavourable growth conditions and to various stress factors (Winter 1951, Dehne 1987). A question arises, whether the endomycorrhizal fungi could also decrease the incidence of free-living saprophytic phytotoxic micromycetes. It has been reported that phytotoxic micromycetes could be an important factor in ARD (Norstadt and McCalla 1968, Berestetskiĭ 1971, Mulder 1974, Čatská *et al.* 1977, 1988).

Materials and methods

Locality and soil type:
1. Degraded chernozem soil from the affected zone of the root system of 70-year-old apple trees cv. Holovouské malinové in the apple orchard - 'apple sick' soil.
2. Degraded chernozem soil from a field near the apple orchard, in which apple trees did not grow for more than 15 years and in which sugar beet was the last crop - 'virgin' soil.
3. Degraded brown soil from the affected zone of the root system of 20-year-old apple nurseries - 'apple sick' soil.
4. Degraded brown soil from a field near the apple orchard, in which apple trees did not grow for more than 15 years and in which wheat was the last crop - 'virgin' soil.

All the soils used are from the Fruit Growing Research and Breeding Institute at Holovousy, north-east Bohemia. The terms 'sick' and 'virgin' soil are used here only in relation to apple trees.

Seedling cultivation and experimental protocol: Stratified surface sterilized (0.1% $HgCl_2$ for 3 min) apple seeds (*Malus domestica* Borkh cv. Kids orange red) incubated for 100 d in wetted sterile sand at 4 °C were sown in garden chernozem soil sterilized by steam and immediately inoculated with endomycorrhizal fungi. After a six-week cultivation in an unheated glasshouse, the seedlings were transferred to containers of 1000 × 400 × 400 mm size (four replications per treatment, *i.e.* each container contained 50 plants) with 'virgin' and/or apple 'sick' soil. Apple seedlings were grown till the end of the experiment (12 months) in an unheated glasshouse without or with inoculation under natural illumination; soil moisture was maintained at 60 % WHC.

Plant growth characteristics: Dry matter was determined by drying samples at 105 °C to constant mass. Leaf area was assessed gravimetrically on leaf replicas.

VAM fungi used for seedlings inoculation: *Glomus fasciculatum* (Thatcher *sensu* Gerd.) Gerd. and Trappe, *Glomus caledonium* (Nicely. and Gerd.) Trappe and Gerd. and *Glomus macrocarpum* Dull. and Dull.

Microbial analysis: The preparation of rhizosphere soil samples, the determination of counts of individual groups of microorganisms, their incubation, and the phytotoxicity test with the alga *Chlorella vulgaris* were performed as described earlier (Čatská *et al.* 1982). A low-nitrogen medium (Rennie 1981) was used for the determination of counts of root-associated diazotrophs (nitrogen-fixing non-symbiotic bacteria).

Results

Incidence of phytotoxic micromycetes and diazotroph bacteria: The inoculation of apple-tree seedlings with some species of the genus *Glomus* influenced the composition of rhizosphere microflora (Table 1). With respect to ARD the incidence in the rhizosphere of phytotoxic micromycetes and diazotroph bacteria after inoculation of apple-tree seedlings by VAM fungi appears to be most important. Besides the type of soil also 'virgin' and/or 'sick' soil used for planting of seedlings were important in the effect of various species of the genus *Glomus* used for inoculation on the rhizosphere microflora. *Glomus fasciculatum* in 'sick' and 'virgin' soil of both types of the soil decreased the amount of phytotoxic micromycetes and increased the amount of diazotroph bacteria. The positive effect on the composition

Table 1. The incidence of phytotoxic micromycetes [% of total micromycetes] and diazotroph bacteria [$\times 10^4$ g^{-1}(soil)] in rhizosphere soil of one-year-old apple seedlings grown in two types of 'sick' (*1* - chernozem soil, *3* - brown soil) and two types of 'virgin' soil (*2* - chernozem soil, *4* - brown soil) after inoculation by VAM fungi - *Glomus fasciculatum* and *G. macrocarpum*. Student's *t*-test: *,** significant differences against the respective controls at $P < 0.05$ and $P < 0.01$, respectively.

Soil	Microorganisms	Without inoculation	Inoculation with *Glomus* *fasciculatum*	*macrocarpum*
1	Phytotoxic micromycetes	45.0	18.2**	34.8**
	Diazotroph bacteria	9.8	16.6**	7.3*
2	Phytotoxic micromycetes	3.1	2.1*	22.0**
	Diazotroph bacteria	16.8	22.2*	14.5
3	Phytotoxic micromycetes	44.0	12.8**	20.8**
	Diazotroph bacteria	7.5	22.5**	17.5**
4	Phytotoxic micromycetes	10.0	1.8**	19.5**
	Diazotroph bacteria	12.8	23.5**	17.3**

of rhizosphere microflora was found especially in 'sick' soil. The possibility to use *Glomus macrocarpum* for inoculation of apple plants was not so clear. In chernozem 'virgin' soil this species increased the amount of phytotoxic micromycetes very significantly. Hence, the choice of a species or strain of VAM fungi for inoculation of plants is equally important as the environmental factors and genera or species or cultivars of plants. If some rhizobacteria as biological control agents are to be widely used in agriculture, they must be compatible with mycorrhizal fungi.

Plant growth and biomass distribution: After the inoculation, growth of apple-tree seedlings improved differently in dependence on the type of soil and on VAM fungus species (Table 2). *G. fasciculatum* positively influenced the plant growth and biomass distribution after 12-month cultivation in both chernozem and brown soils, whereas *G. macrocarpum* only in brown soil. This effect was much more evident in the soil with ARD (Table 2).

Table 2. Growth of one year old apple-tree seedlings in two types of 'sick' soil and two types of 'virgin' soil without inoculation (C) and after inoculation by VAM fungi - *Glomus fasciculatum* (*G.f.*) and *G macrocarpum* (*G.m.*). Student's *t*-test: *,** significant differences against the respective controls at $P < 0.05$ and $P < 0.01$, respectively.

'Sick' chernozem			'Virgin' chernozem			'Sick' brown soil			'Virgin' brown soil		
C	*G.f.*	*G.m.*	C	*G.f.*	*G.m.*	C	*G.f.*	*G.m.*	C	*G.f.*	*G.m.*
Plant height [mm]											
102	171**	88**	255	293**	221*	122	203**	175*	234	285**	250*
Shoot fresh mass [g]											
2.73	4.65**	2.32	6.39	7.00*	5.41*	2.96	5.40**	3.99*	6.21	6.89	5.68
Root fresh mass [g]											
2.25	3.49**	1.92	5.95	6.20*	4.33	2.57	3.68*	3.52*	5.30	6.15	4.85
Shoot dry mass [g]											
0.94	1.62	0.85	2.20	2.44	1.98	1.02	1.88**	1.46*	2.14	2.40*	2.08
Root dry matter [g]											
0.70	1.30**	0.62	1.84	2.10*	1.60	0.80	1.56**	1.30*	1.65	2.04*	1.79

Discussion

Soil and rhizosphere microorganisms can be included in the complex of factors which can influence the occurrence and efficiency of natural mycorrhizal associations as well as of those mycorrhizal fungi which can be used for the inoculation of plants. By the inoculation of species of endomycorrhizal fungi it is possible to stimulate the growth of apple seedlings (Čatská and Vejsadová, unpublished). The composition of the rhizosphere microflora is related with growth of plants. The growth is better when the amount of phytotoxic micromycetes is lower and the amount of diazotroph bacteria is higher due to inoculation by VAM fungi.

Before trying to regulate the all processes that could positively affect plant growth and health of plants, it was necessary to accumulate information about interactions among individual components of the rhizosphere free-living microorganisms and VAM fungi and between these organisms and plants. Some authors reported antagonism (Davis *et al.* 1978, Krishna *et al.* 1982, Paulitz and Linderman 1989) and other authors described synergism (Barea *et al.* 1983, Meyer and Linderman 1986, Azcón 1989) between VAM fungi and other free-living rhizosphere microorganisms. It is known that the rhizosphere microflora (especially bacteria of the genera

Rhizobium, Azotobacter, Pseudomonas, Azospirillum) can affect the formation of VAM fungi (Will and Sylvia 1990). On the other hand, the results of our experiments show that VAM fungi can affect the composition of the rhizosphere microflora. The so-called mycorhizosphere effect has been reviewed by Linderman (1988) and Paulitz and Linderman (1989). Our previous experiments brought evidence that the ARD may be caused by saprophytic phytotoxic micromycetes (Čatská *et al.* 1988). These phytotoxic micromycetes affect negatively the other rhizosphere microflora and especially the growth and health of plants. The inoculation of apple seedlings with some species of the genus *Glomus* decreased the amount of these phytotoxic micromycetes and, on the other hand, increased the amount of diazotroph bacteria. These bacteria could also serve as antagonists against phytotoxic fungi (Čatská and Hudská 1993). The growth-promoting role of this group of bacteria for plants consists more in the production of antibiotics and phytohormones than in nitrogen fixation under European conditions.

It follows from the results obtained that VAM fungi can favourably affect the plants directly and also indirectly by means of the rhizosphere microflora. VAM fungi can change the composition of the rhizosphere microflora and this can be important particularly in the monocultural rhizosphere where the equilibrium between favourable and harmful microorganisms is impaired. It also appears that the choice of species or strains of VAM fungi for inoculation of plants depends more on environmental factors (Plenchette *et al.* 1982, Pacovsky *et al.* 1985), than on the genera or species or cultivars of plants as described in the literature.

The utilization of VAM fungi, either alone or together with some rhizosphere microorganisms, especially with plant growth promoting bacteria, is thus even more important in monocultural conditions than in the crop rotation system.

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