Mycorrhizae Protects Plant Roots from Pathogens:

Abstracts of Research

Direct interaction between the arbuscular mycorrhizal fungus *Glomus intraradices* and different rhizosphere microorganisms

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Arbuscular mycorrhizal (AM) fungi can reduce the incidence and importance of plant root diseases caused by pathogens. The mechanisms involved are not well characterized. We used an *in vitro* experimental system to test the hypothesis that the extraradical mycelium of AM fungi can interfere directly with microorganisms in the mycosphere and directly or indirectly reduce the population of plant pathogens. This system permitted the isolation of soluble substances released by the extraradical mycelium of *Glomus intraradices*. The AM fungus was grown on *Daucus carota* transformed roots in one compartment, while only the extraradical mycelium was allowed to grow in a second compartment. A freezing and centrifugation technique was developed for the extraction and concentration of substances present in the compartment containing only the AM fungal mycelium. Four soil-inhabiting microorganisms were selected, and conidial germination (fungi) or growth (bacteria) of these was studied in the presence and absence (control) of the extract. In comparison with the control, the results indicated that both the growth of *Pseudomonas chlororaphis* and the conidial germination of *Trichoderma harzianum* were stimulated in the presence of the AM fungal extract. In contrast, **conidial germination of *Fusarium oxysporum* f. sp. *chrysanthemi* was reduced** while the growth of *Clavibacter michiganensis* subsp. *michiganensis* was not affected. The measured effects in general were directly correlated with extract concentration. Differences in pH were noted between the extract containing substances released by the AM fungus and the non-AM control, but no significant influence of the pH on growth or conidial germination was noted, confirming that substances released by the

AM fungus in the growth medium is the main factor explaining differential growth of the microorganisms tested. The results suggest that direct interactions exist between AM fungi and soil microorganisms, which might lead to changes in microbial equilibrium detrimental to pathogens.

Colonization patterns of root tissues by *Phytophthora nicotianae* var. *parasitica* related to reduced disease in mycorrhizal tomato

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Abstract Tomato plants pre-colonised by the arbuscular mycorrhizal fungus*Glomus mosseae* showed decreased root damage by the pathogen*Phytophthora nicotianae* var.*parasitica*. In analyses of the cellular bases of their bioprotective effect, a prerequisite for cytological investigations of tissue interactions between*G. mosseae* and*P. nicotianae* v.*parasitica* was to discriminate between the hyphae of the two fungi within root tissues. We report the use of antibodies as useful tools, in the absence of an appropriate stain for distinguishing hyphae of*P. nicotianae* v.*parasitica* from those of*G. mosseae* inside roots, and present observations on the colonisation patterns by the pathogenic fungus alone or during interactions in mycorrhizal roots. Infection intensity of the pathogen, estimated using an immunoenzyme labelling technique on whole root fragments, was lower in mycorrhizal roots. Immunogold labelling of*P. nicotianae* v.*parasitica* on cross-sections of infected tomato roots showed that inter or intracellular hyphae developed mainly in the cortex, and their presence induced necrosis of host cells, the wall and contents of which showed a strong autofluorescence in reaction to the pathogen. In dual fungal infections of tomato root systems, hyphae of the symbiont and the pathogen were in most cases in different root regions, but they could also be observed in the same root tissues. The number of*P. nicotianae* v.*parasitica* hyphae growing in the root cortex was greatly reduced in mycorrhizal root systems, and in mycorrhizal tissues infected by the pathogen, arbuscule-containing cells surrounded by intercellular*P. nicotianae* v.*parasitica* hyphae did not necrose and only a weak autofluorescence was associated with the host cells. Results

are discussed in relation to possible processes involved in the phenomenon of bioprotection in arbuscular mycorrhizal plants.

The use of arbuscular mycorrhizae to control onion white rot (Sclerotium cepivorum Berk.) under field conditions

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A field experiment was carried out to determine the effects of the inoculation of onion (Allium cepa L.) with Glomus sp. Zac-19 on the development of onion white rot (Sclerotium cepivorum Berk.) and on onion production. **Mycorrhization delayed onion white rot epidemics by 2 weeks and provided a significant protection against the disease** for 11 weeks after onion transplanting, as compared with nonmycorrhizal controls. **Mycorrhizal plants showed an increase of 22% in yield, regardless of the presence of the white rot pathogen.**

Mechanism of control of root-feeding nematodes by mycorrhizal fungi in the dune grass Ammophila arenaria

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de la Pena E, Echeverria SR, van der Putten WH, Freitas H, Moens M.

Root-feeding herbivores can affect plant performance and the composition of natural plant communities, but there is little information about the mechanisms that control root herbivores in natural systems. This study explores the interactions between the pioneer dune grass Ammophila arenaria, arbuscular mycorrhizal fungi (AMF) and the root-feeding nematode Pratylenchus penetrans. Our objectives were to determine whether AMF can

suppress nematode infection and reproduction and to explore the mechanisms of nematode control by AMF. A sequential inoculation experiment and a split-root experiment were designed to analyse the importance of plant tolerance and resistance and of direct competition between AMF and P. penetrans for the root herbivore and the plant. Root infection and multiplication of P. penetrans were significantly reduced by the native inoculum of AMF. **Plant pre-inoculation with AMF further decreased nematode colonization and reproduction**. Nematode suppression by AMF did not occur through a systemic plant response but through local mechanisms. **Our results suggest that AMF are crucial for the control of root-feeding nematodes in natural systems and illustrate that locally operating mechanisms are involved in this process**.

Growth response of the plum rootstock AD 101 to mycorrhizal inoculation with *Glomus mosseae* and *Glomus intraradices* in a replant soil infested with nematodes

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Micropropagated plants of the plum (*Prunus insititia*) rootstock AD 101 adapted to heavy and calcareous soils were inoculated with *Glomus mosseae* and *Glomus intraradices* in a pasteurized sandy soil-sphagnum peat mixture (5:1,v/v). Plant development, measured as height and stem diameter, was significantly increased by both mycorrhizal inoculation treatments compared with noninoculated plants after 6, 9 and 18 months growth. When plants were 18 months-old, they were transplanted to a replant soil infested with nematodes, pasteurized and not pasteurized, under open air microplot conditions, and harvested 9 months later. *Glomus intraradices* achieved a higher percentage of root colonization than *G. mosseae* in pasteurized replant soil, but both inoculation treatments were still significantly effective at increasing plant biomass 30 months after plant inoculation. When the replant soil was not pasteurized, native endophytes, although being less infective and less effective at increasing plant development than *G. intraradices,* colonized plant roots. **The number of nematodes per gram root was significantly lower in plants inoculated with both Glomus species than in plants naturally infected**.

Inhibition of Fusarium oxysporum f. sp. dianthi in the non-VAM species Dianthus caryophyllus by co-culture with Tagetes patula companion plants colonized by Glomus intraradices

StArnaud, M; Hamel, C; Vimard, B; Caron, M; Fortin, JA. 1997. Can. J. Bot.-Rev. Can. Bot. 75(6): 998-1005.

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The effect of the mycorrhizal fungus Glomus intraradices on disease development caused by Fusarium oxysporum f.sp. dianthi in the nonmycorrhizal species Dianthus caryophyllus was studied by co-culture of carnation plants with the mycorrhizal species Tagetes patula. Presence of VAM T. patula plants more than doubled the survival of D. caryophyllus, significantly reduced the disease symptoms, and decreased Fusarium oxysporum dianthi propagules by 4:1 in soil. Non-VAM T. patula plants had no effect. Dianthus caryophyllus shoot biomass was reduced by Fusarium oxysporum dianthi in non-VAM controls but was not affected in presence of Glomus intraradices. Glomus intraradices alone has no effect on T. patula or D. caryophyllus shoot biomass. Dianthus caryophyllus mineral shoot content was not modified by G. intraradices. In absence of T. patula, G. intraradices did not colonize D. caryophyllus, whereas in its presence, 14-20% of the carnation root length contained abundant vesicles and hypha but very rarely arbuscules. **The presence of G. intraradices clearly reduced the disease caused by Fusarium oxysporum dianthi in D. caryophyllus**. Reduction in disease severity was associated with reduced F. o. dianthi propagule number in the substrate and was clearly unrelated to plant nutrition. Our results may be explained either by the induction of D. caryophyllus disease resistance mechanisms by the mycorrhizal fungus or by direct or indirect microbial interactions in the soil.

FUSARIUM CROWN AND ROOT-ROT OF TOMATO IN FLORIDA USING TRICHODERMA-HARZIANUM AND GLOMUS INTRARADICES

BIOLOGICAL CONTROL 5: 427-431.

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Field experiments were conducted to evaluate commercial formulations of two beneficial fungi, Trichoderma harzianum and Glomus intraradices, for the control of Fusarium crown and root rot of tomato, caused by Fusarium oxysporum f. sp. radicis-lycopersici. Tomato seeds cv. "Sunny" were planted into soil nonamended or amended with the biocontrol agents, the former treatment serving as the control. After 6-7 weeks, plants were transplanted into beds fumigated with methyl bromide-chloropicrin in commercial tomato fields with a previous history of Fusarium crown and root rot. Disease incidence and severity were recorded at harvest maturity. Large and extra-large fruits (greater than or equal to 6.27 cm) also were harvested, counted, and weighed at maturity. Total marketable fruit yield was also determined, Compared to the controls, significant decreases in disease incidence were obtained with treatments of T. harzianum (1993), G. intraradices (1991), and T. harzianum + G. intraradices (both years). Significant decreases in disease severity were obtained with the treatments of T. harzianum (1993), G. intraradices (1991), and T. harzianum + G. intraradices (1993), Yields of large and extra-large fruit or total marketable yield were not significantly different over the controls. **These data suggest that commercial biological control agents are effective in reducing Fusarium crown and root rot and that further evaluation of these agents is justified.**

Effects of Glomus aggregatum on lethal yellowing disease of Java citronella caused by Pythium aphanidermatum

Ratti, N; Alam, M; Sharma, S; Janardhanan, KK. 1998. SYMBIOSIS 24(1):115-126.

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Effects of the vesicular-ARBUSCULAR Mycorrhiza (VAM), Glomus aggregatum Schenck and Smith emend Koske on the lethal yellowing disease of Java citronella (Cymbopogon winterianus Jowitt) caused by Pythium aphanidermatum (Edson) Fitzp. and its interaction with the pathogen affecting plant growth, biomass production, N, P and K concentrations and acid phosphatase activity were investigated under glasshouse conditions. Citronella plants infected by P. aphanidermatum (Pa treatment) were chlorotic and showed a reduction in biomass production from 39.8 to 18.2 g plant (-1), N, P and K concentrations in shoot from 14.7, 3.51 and 12.7 to 13.3, 1.74 and 5.4 mg g (-1) dry weight, respectively, and acid phosphatase activity from 17.52 to 11.08 mu m p-nitrophenol min (-1) mg (-1) fresh weight over untreated healthy control plants. Treatments of 15dGa+Pa and simGa+Pa reduced lethal yellowing by 80% and 60%, respectively as compared with Pa treatment. Further, 15dGa+Pa treatment increased the biomass by 163.74%, N, P and K concentrations in shoot by 51.88%, 152.29% and 157.41% and acid phosphatase activity in root by 80.96%, respectively, as compared with Pa treatment. Colonization by the VAM fungus (Ga treatment) also enhanced biomass, N, P and K concentrations and acid phosphatase activity over the non VAM control. **It is concluded that G. aggregatum improves the biomass production and reduces the damaging effect of P. aphanidermatum on Java citronella**.

Effectiveness of Glomus species in protecting white clover against nematode damage

Habte, M; Zhang, YC; Schmitt, DP. 1999. Can. J. Bot.-Rev. Can. Bot.7: 135-139. Three species of arbuscular mycorrhizal (AM) fungi, Glomus aggregatum

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Three species of arbuscular mycorrhizal (AM) fungi, Glomus aggregatum Schenck and Smith emend. Koske, Glomus intraradices Schenck and Smith, and Glomus mosseae (Nicol. and Gerd.) Gerdemann and Trappe, were evaluated for their effectiveness to suppress the plant parasitic nematode Meloidogyne incognita (Kofoid and White) Chitwood in white clover (Trifolium repens L.) in a greenhouse study. Forty 1-day-old seedlings not colonized or colonized to the same extent by the fungi were transplanted into a growth medium uninoculated or inoculated with 10 000 M. incognita eggs. After 40 days of further growth, root mass, shoot mass, extent of galling, number of nematodes and nematode eggs in roots, and extent of mycorrhizal colonization of roots were determined. **Growth of white clover was significantly stimulated by mycorrhizal colonization, and**

nematodes caused the greatest damage when plants were not colonized by the fungi. The degree to which mycorrhizal fungi reduced nematode damage varied with the species of mycorrhizal fungus; the extent of damage reduction ranged from 19 to 49.8%, based on loss of shoot mass.

Effect of two vesicular-arbuscular mycorrhizal fungi on the growth of micro-propagated potato plantlets and on the extent of disease caused by Rhizoctonia solani

Yao MK; Tweddell RJ; Desilets H. 2002.. MYCORRHIZA. 12(5)235-242.

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Two micro-propagated potato cultivars, Goldrush and LP89221, were inoculated into sowing trays with either Glomus etunicatum or G. intraradices in a greenhouse. After 2 weeks, plantlets were transplanted into pots and roots were challenged 7 days later with Rhizoctonia solani. At different times after R. solani infection, disease severity, mortality rate, root colonization levels, various growth parameters, and shoot mineral content were evaluated. In Goldrush, only inoculation with G. etunicatum led to a significant reduction in disease severity, ranging between 60.2% and 71.2%, on both shoot and crown. This decrease was not observed in LP89221. Compared with the control plantlets, **inoculation of Goldrush with G. etunicatum or G. intraradices reduced significantly the mortality rate by 77% and 26%, respectively**, whereas vesicular-arbuscular mycorrhizal (VAM) fungi did not significantly influence the mortality rate in LP89221. **In Goldrush, inoculation with G. etunicatum significantly increased shoot fresh weight, root dry weight and the number of tubers produced per plant, whereas G. intraradices only significantly increased the number of tubers**. Tuber and root fresh weights of both potato cultivars were significantly reduced by R. solani infection. However, R. solani-infected plantlets of both Goldrush and LP89221, inoculated with G. etunicatum, produced significantly greater tuber fresh weight than non-VAM plantlets. In R. solani-infected plantlets of Goldrush but not LP89221, G. etunicatum and G. intraradices increased root fresh weight by approximately 140.3% and 76.5%, respectively, compared with non-AM plants.

Effect of arbuscular mycorrhiza fungi (AMF) on Nematode - *Meloidogyne hapla* in pyrethrum in Kenya

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Two arbuscular mycorrhiza fungi (AMF) isolated from a pyrethrum growing region in Kenya were screened for efficacy against a nematode, *Meloidogyne hapla* in the greenhouse. The fungi were identified at INVAM (International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi) as *Glomus etunicatum* (Isolate KS18) and *Glomus* sp. (Isolate KS14). **Isolate KS14 (*Glomus* sp.) significantly suppressed nematode population, growth and development by up to 54%, egg production by up to 75% and disease severity by up to 71%. *Glomus etunicatum* (Isolate KS18) suppressed nematode growth and development by up to 50%, egg production by up to 75% and disease severity by up to 57%.** In addition, *G. etunicatum and* Isolate KS14, significantly improved top dry biomass of pyrethrum by up to 33% and 47%, respectively.

Growth enhancement and verticillium wilt control by vesicular-arbuscular mycorrhizal fungus inoculation in eggplant

Matsubara Y; Tamura H; Harada T. 1995.. JOURNAL OF THE JAPANESE SOCIETY FOR HORTICULTURAL SCIENCE. 64(3):555-561.

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Eggplant (Solanum melongena L.) plantlets infected with vesicular-arbuscular mycorrhizal (VAM) fungus (Glomus etunicatum and Gigaspora margarita) were transplanted to a field contaminated with Verticillium dahliae to investigate the disease tolerance of a VAM fungus-infected plant. 1. Eight weeks after transplanting, plant height, number of leaves,

leaf size, and proximal diameter of the main stem were larger in VAM fungus-infected plants than in non-inoculated plants. The primary flower bloomed later in VAM fungus-infected plants than in non-inoculated plants. 2. The rate of VAM fungus-infected portions in a whole root system reached the maximum of 40.8% with Glomus etunicatum 10 weeks after inoculation, whereas it was 40.2% with Gigaspora margarita 8 weeks later. 3. **The appearance of verticillium wilt was delayed and reduced by VAM fungus-infection, and the effects were more apparent in Glomus etunicatum than in Gigaspora margarita.** VAM fungus-infected plants yielded more fruits than did the non-inoculated plants; the incidence of fruit malformation caused by verticillium wilt were smaller, especially in Glomus etunicatum-infected plants. 4. Lignin (stained with a reagent consisting of phloroglucinol and HCl) accumulated in the firstly and secondly branched roots more richly in VAM fungus-infected plants than in non-inoculated plants. Observation using transmission electron microscope (TEM) revealed that the secondary cell wall of cortical cells in the thirdly branched roots was thicker in VAM fungus-infected plants than in non-inoculated plants.