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Effects of the root-lesion nematode *Pratylenchus vulnus* and the mycorrhizal fungus *Glomus mosseae* on the growth of EMLA-26 apple rootstock

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Abstract. The interaction between *Pratylenchus vulnus* and the endomycorrhizal fungus *Glomus mosseae* on the growth of EMLA 26 apple rootstock was studied under shadehouse conditions in the field during the first 6 months of growth. Fresh top weights, fresh root weights, and shoot lengths of mycorrhizal plants with and without *P. vulnus* were significantly higher than those of nonmycorrhizal plants. Addition of P to nonmycorrhizal controls had little overall effect. Mycorrhizal treatments with the nematode showed a significantly lower amount of nematodes per gram of root than nonmycorrhizal treatments with *P. vulnus*. Root colonization by *G. mosseae* was not affected by the presence of the nematode. No nutrient deficiencies were detected in foliar analyses, although low levels of K, Al, and Fe were detected in nematode treatments. The highest levels of S, Mg, Mn and Zn were detected in *P. vulnus* inoculated plants. Mycorrhizal plants had the highest levels of N, Na, P, K, and Fe. The importance of early mycorrhizal infection of EMLA 26 apple rootstock in the presence of the nematode is discussed.

Key words: Apple – *Glomus mosseae* – Interaction – *Pratylenchus vulnus* – Vesicular-arbuscular mycorrhizae

Introduction

The lesion-nematode *Pratylenchus vulnus* Allen and Jensen is an important pest attacking several stone fruit, pome fruit and nut tree crops throughout the world (McElroy 1972; Lamberti 1981; Scotto La Massese 1989). This nematode is present in Spain in nurseries and commercial orchards. It has been found to be pathogenic in apple, pear (Fernández et al. 1992) and several *Prunus* rootstocks (Pinochet et al. 1991) recently introduced into the country.

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Arbuscular mycorrhizal fungi (AM) are associated with virtually all fruit tree species and this normally occurs naturally in the nursery (early infection) or when transplanted to the field. AM fungi are obligate symbionts which increase nutrient uptake by plants, especially P (Gerdemann 1968; Smith 1987). They have been shown to benefit plants under physiological stress, such as during drought (Nelson 1987), in nutrient deficient soils (Linderman 1988), and when attacked by pests and diseases (Dehne 1982; Perrin 1991).

The high susceptibility of the apple rootstock EMLA 26 to *P. vulnus* (Fernández et al. 1992) and the mycorrhizal dependency of apple rootstocks (Plenchette et al. 1981; Hoepfner et al. 1983) encourage studies of whether early AM colonization of micropropagated plant material confers some degree of protection when established in infested soils at a stage when plants are most vulnerable. This has been observed with mycorrhizal rooted cuttings of plum inoculated with *P. vulnus* (Camprubi et al. 1993) and reported with mycorrhizal citrus seedlings infected with another migratory, endoparasitic nematode, *Radophulus citrophilus* (O'Bannon and Nemec 1979).

The effects of AM fungi on *P. vulnus*-infected young apple plants is unknown but they may result in an increased nutritional capacity of the plant in the presence of the nematode. The purpose of this investigation was to study the effects of *P. vulnus* on plant growth and nutrition in plantlets of EMLA 26 apple rootstock inoculated or not inoculated with the AM fungus *Glomus mosseae* (Nicol and Gerd.) Gerd. and Trappe.

Materials and methods

Micropropagated apple (*Malus silvestris* L.) rootstock EMLA-26 was obtained from Agromillora Catalana S.A., Sant Sadurní d'Anoia, Barcelona, Spain. Climatized *in vitro* plantlets with incipient root growth in 50 ml minipots were transferred to 300-ml pots with a pasteurized 4:1:1 (v:v:v) sandy soil-sphagnum peat-

quartz sand mixture. During transplant, the mycorrhizal treatments were inoculated with 13 g of *G. mosseae* soil inoculum. The inoculum consisted of rhizosphere soil from onion (*Allium cepa* L.) and clover (*Trifolium repens* L.) pot cultures with a density of 20 sporocarps/g. The plants not receiving mycorrhizal inoculum received a filtrate of soil inoculum free of AM propagules. Two months later, plants were transferred to 5.2-l containers filled with a sandy textured soil (83% sand, 14% silt, 2% clay), at pH 7.52, and with less than 2% organic matter, and kept in a greenhouse for 1 week prior to nematode inoculation. The phosphorus content of the soil at transplant was 9–10 ppm.

Six treatments were established: 1) control (without nematode or *G. mosseae*); 2) plants inoculated with *P. vulnus*; 3) plants inoculated with *G. mosseae*; 4) plants with joint inoculations of *G. mosseae* and *P. vulnus*; 5) control with P fertilization; 6) control with P fertilization and *P. vulnus*.

A *P. vulnus* population isolated from rose (*Rosa multiflora* L.) in Cabrils, Barcelona, was cultured monoxenically on carrot disks (Moody et al. 1973). The identification to the species level was made by the Commonwealth Institute of Parasitology, St. Albans, UK. Inoculum was recovered from stock cultures by adding sterile water and collecting the nematodes with a pipette. The volume of the nematode suspension was adjusted to give 500 individuals per plant and delivered through 4 holes located at a distance of 4–5 cm from the base of the stem. Containers were laid out in a bucket microplot set-up (Barker 1985). These were buried in sand 80 cm apart in an open shadehouse in field conditions until the conclusion of the study after 5 months. During the course of the study, the shadehouse temperature fluctuated between 14 and 31° C. Plants were watered as needed and fertilized weekly with a modified Hoagland's nutrient solution lacking phosphorus (Hoagland and Arnon 1950), except for two treatments in which P was included (0.136 g KH_2PO_4/l H_2O). Each treatment was replicated ten times in a completely randomized design. Nematode reproduction and plant growth (fresh top weight, fresh root weight, shoot diameter and shoot length) were assessed 150 days after inoculation.

Nematodes in soil were recovered by differential sieving and sugar flotation (Jenkins 1964) from an homogenized 250-ml subsample. Nematodes in roots were extracted by cutting the whole root system into 1-cm-long pieces and macerating them with water in a commercial blender for 30 s at 10-s intervals. The nematode suspension was then concentrated using 0.15-, 0.074- and 0.025-mm sieves (100, 200 and 500 mesh, respectively). Root tissue and debris collected on the 0.150-mm sieve were discarded.

The data for top weights, root weights, shoot diameter, and shoot length were analyzed by a one-way analysis of variance. Final nematode populations and nematodes per gram of root were transformed to $\log_{10}(x+1)$ for analysis. Means were compared by Tukey's multiple range test ($P \leq 0.05$).

For assessing mycorrhizal infection, root samples were collected and stained with 0.05% trypan blue in lactic acid (Phillips and Hayman 1970) modified by the procedure described by Koske and Gemma (1989). The percentage of root colonization was determined using the grid line intersect method (Giovanetti and Mosse 1980).

Macro and micro elements were determined at harrest time. For this, composite leaf samples from 5 to 6 leaves were taken starting from the lowest normal leaf on the shoot, avoiding senescent or necrotic tissue and leaves free of surface contamination by applied nutrient solution. Leaves were thoroughly washed in distilled water and prepared for analysis by dehydration in a temperature-controlled, fan-ventilated oven at 70° C ± 1 for 48 h. Samples were ground in a ball mill and digested in wet acid (Jones et al. 1991) using nitric and perchloric acid. Analysis of all elements except N was made with a Thermo Jarrell Ash inductively coupled plasma (ICP) emission spectrometry (Munter and Grande 1981). N content was determined with a Carlo Erba NA 1500 gas chromatograph.

To observe possible interactions between AM fungi and nematodes within the root cortex, selected pieces of roots from the different treatments were examined by scanning electron microscope (SEM). For SEM observation, the cellular contents of root tissue were digested, dehydrated to critical point in CO_2, mounted, sputtercoated with gold and examined at accelerating potentials of 15 kV.

Results

Fresh top weights of *P. vulnus*-inoculated apple plantlets were relatively low and differed significantly from the *G. mosseae* and *G. mosseae* + *P. vulnus* treatments (Table 1). Plants inoculated with *G. mosseae* + *P. vulnus* had a significantly larger shoot diameter than *P. vulnus*-inoculated plants. *G. mosseae*-inoculated plants attained the highest shoot length values and differed from the *P. vulnus* and control with P treatments, but not from the others. Fresh root weights of both mycorrhizal EMLA 26 (with and without *P. vulnus*) were significantly higher than *P. vulnus* alone and *P. vulnus* with P inoculated plants. There were no differences between control, control with P and *G. mosseae* treatments for fresh root weights. At harvest, the mycorrhizal treatments with and without the nematode showed a similar percentage of root colonization: *G. mosseae* (40%) versus *G. mosseae* + *P. vulnus* (37%).

In plants inoculated with *P. vulnus*, there were no differences between treatments in the final nematode population. However, mycorrhizal EMLA 26 with the nematode showed a significantly lower amount of nematodes per gram of root compared to nonmycorrhizal treatments (Table 2).

Table 1. Top weights, shoot lengths, shoot diameters, and root weights of EMLA 26 apple rootstock evaluated under shadehouse conditions with 500 *Pratylenchus vulnus* per plant in combination with *Glomus mosseae* and P fertilization 5 months after nematode inoculation. The data are the means of 10 replicates. Means in the same column followed by the same letter do not differ according to Tukey's multiple range test ($P \leq 0.05$)

Treatment	Fresh top weight (g)	Shoot diameter (mm)	Shoot length (cm)	Fresh root weight (g)	Percentage AM colonization
Control	5.12 ab	4.75 ab	14.92 abc	9.52 abc	—
Control + P	4.36 ab	4.54 ab	13.55 ab	7.61 ab	—
P. vulnus	2.65 a	4.16 a	8.84 a	5.68 a	—
P. vulnus + P	4.46 ab	4.42 ab	16.18 abc	4.84 a	—
G. mosseae	7.62 b	4.48 ab	23.94 c	12.19 bc	40
G. mosseae + *P. vulnus*	7.97 b	5.43 b	19.12 bc	13.77 c	37

Table 2. The reproduction of *Pratylenchus vulnus* on EMLA 26 apple rootstocks in combination with *Glomus mosseae* and P fertilization 5 months after inoculation with 500 nematodes per plant. The data are the means of 10 replicates. Actual data are presented but the data were transformed to $\log_{10}(n+1)$ for analysis. Means in the same column followed by the same letter do not differ according to Tukey's multiple range test ($P \leq 0.05$). Pf/Pi, Final population/initial population (nematode reproduction rate)

Treatment	Final population per plant (soil and roots)	Nematodes per g root	Pf/Pi
P. vulnus alone	122310 a	18350 a	245
P. vulnus+P	151470 a	24380 a	303
P. vulnus+*G. mosseae*	164980 a	8980 b	330

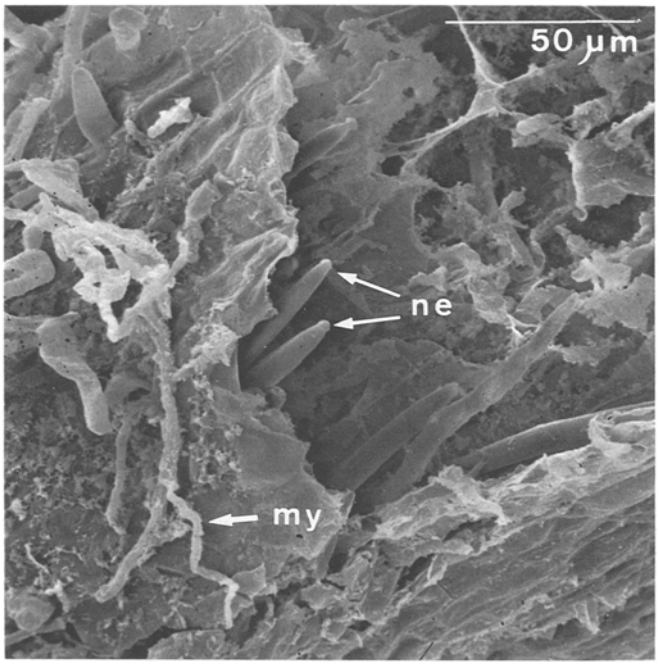

Fig. 1. The root-lesion nematode *Pratylenchus vulnus* (*ne*) and mycelium (*my*) of *Glomus mosseae* colonizing the cortical parenchyma of a root of EMLA 26 apple rootstock

No deficiencies were detected by foliar analysis in any of the treatments, although low levels for apple were found for some elements in some treatments (Table 3). Leaves of plants inoculated with the nematode were low in K, Al, and Fe. Treatments with P in combination with the nematode were low in N, Ca, K, and Cu. However, S, Mg, Mn, and Zn reached the highest values in *P. vulnus*-inoculated plants. Levels of P were low in general, except for plants inoculated with *G. mosseae*, for which levels were normal. *G. mosseae*-inoculated plants also achieved the highest values for N, Na, K, and Fe.

SEM observations revealed extensive colonization of *P. vulnus* in the cortical parenchyma. *G. mosseae* (mycelium) was found together with the nematode colonizing the same tissue (Fig. 1). Both parasite and symbiont were generally found in young, actively growing roots.

Discussion

In a nutritional study that included AM, low and high P treatments, Smith and Kaplan (1988) concluded that increased plant growth and fewer citrus-burrowing nematodes in mycorrhizal and high P plants were direct results of increased P nutrition of the host. In this study, the addition of P had no overall effect on plant growth or nematode multiplication in either *P. vulnus*-inoculated on non-inoculated plants, although P levels were lower that those applied by Smith and Kaplan (1988) to citrus. Plants with *P. vulnus* and P showed the highest numbers of nematodes per gram of root (24380).

P. vulnus mycorrhizal plants grew significantly more than nematode-inoculated plants without mycorrhiza, and had a significantly lower number of nematodes per gram of root (8980) than nonmycorrhizal plants inoculated with *P. vulnus* alone (18350) or *P. vulnus* with phosphorus (24380), suggesting that root colonization by *G. mosseae* has a negative effect on the development of *P. vulnus*. However, at such high levels of nematode parasitism on apple roots, the possible prophylactic effect of mycorrhizae on the nematode colonization would seem minor. In a previous AM-nematode interaction study with this same nematode on three plum rootstocks, *G. mosseae* had no detrimental effect on *P. vulnus* when compared to *P. vulnus*-inocu-

Table 3. Mineral constituents of composite leaf samples from apple rootstock EMLA 26 inoculated with *Pratylenchus vulnus* and *Glomus mosseae* alone and in combination 5 months after inoculation with the nematode

Treatment	Percentage dry weight							ppm				
	N	Ca	Mg	Na	K	S	P	Al	Fe	Mn	Cu	Zn
Control	1.58	1.51	0.38	0.02	1.30	0.14	0.08	318	250	376	4.42	65.4
Control+P	1.68	1.25	0.34	0.04	1.44	0.12	0.06	408	332	235	—	74.6
P. vulnus	1.71	1.66	0.38	0.03	1.07	0.15	0.07	308	225	440	3.98	89.8
P. vulnus+P	1.48	0.96	0.31	0.03	1.20	0.14	0.08	507	329	206	2.66	36.2
G. mosseae	1.92	1.13	0.33	0.04	1.57	0.14	0.13	565	354	331	4.01	56.9
G. mosseae+*P. vulnus*	1.83	1.18	0.35	0.02	1.14	0.14	0.08	326	241	225	3.15	51.8

lated plants without *G. mosseae* (Camprubi et al. 1993). These conflicting results suggest that the ability of this nematode to colonize root tissue in areas where the AM fungus is already established depends on the suitability of the host for the nematode.

The percentage of root colonized in mycorrhizal plants did not vary in the presence or absence of the nematode (40 and 37%, respectively), indicating that feeding and migration of *P. vulnus* in cortical root tissues did not have a direct negative effect on *G. mosseae* colonization during the first 5 months after nematode exposure. However, these data should be interpreted with caution since *P. vulnus* is highly destructive on apple (Fernández et al. 1992). A longer period of exposure to the nematode might reduce feeding sites for fungal growth. The level of mycorrhizal colonization obtained in our study is similar to that obtained by Granger et al. (1983) with EMLA 111 and EMLA 7 apple rootstocks 15 weeks after inoculation in nematode-free soil.

In a nutritional study with *P. vulnus* on rose (Sher 1957) conducted under greenhouse conditions and lasting 1 year, *P. vulnus* treatments showed marked reductions in K, Ca, Cu, and F by leaf analysis, with visible deficiency symptoms. The treatments also resulted in increased Zn and Mg levels. Culver et al. (1989) found that *P. vulnus* infection of rootstocks caused significant decreases in K and Ca contents of leaves on Nemaguard peach, and increased Mg on *Pistacia atlantica* 122 days after inoculation with the nematode. Results obtained in our study with EMLA-26 apple rootstock with the nematode alone or in combination with P showed the same pattern for these elements. This suggests that absorption and transport of some elements to aerial parts are impaired by destruction of the root cortical tissues caused by the nematode, probably due to loss of the ability for differential permeability and thus reduced nutrient element transport (Kirkpatrick 1964). In contrast other elements, especially Zn, Mg, and Mn, are absorbed continuously and accumulate in leaf tissues as a result of reduced growth. Particularly interesting is the fact that the majority of mineral elements in mycorrhizal *P. vulnus* plants, with the exception of Na, were less affected (near normal values). Such plants grew well, similar to plants with *G. mosseae* alone.

It is noteworthy that plants seriously affected by the nematode infection showed an intense green coloration in nearly all the replicates. The leaves of these plants were small and curled backwards. Several plants in th P+nematode treatment showed similar symptoms suggesting that the phenomenom is related to the nematode.

It is concluded that EMLA 26 responds favourably to mycorrhizal infection in both the absence and the presence of *P. vulnus*, conferring a significant protective effect against this pathogen and an increased capacity for nutrient uptake; this is reflected in increased plant growth and lower nematode densities per gram of root. From the practical standpoint, early mycorrhizal inoculation of nursery plant material could be important for establishing trees in nematode-infested soil at a stage when plants are most vulnerable.

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