Inducing Tolerance to the Root-lesion Nematode *Pratylenchus vulnus* by Early Mycorrhizal Inoculation of Micropropagated Myrobalan 29 C Plum Rootstock

J. Pinochet, A. Camprubí, C. Calvet, and C. Fernández

Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Departament de Patologia Vegetal, Centre de Cabrils, Crta. de Cabrils s/n, 08348 Cabrils, Barcelona, Spain

R. Rodríguez Kábana

Department of Plant Pathology, Auburn University, Auburn, AL 36849

ADDITIONAL INDEX WORDS. arbuscular mycorrhizal fungi, *Glomus intraradices*, *Glomus mosseae*, mycorrhizae, *Prunus cerasifera* × *P. munsoniana*, protection

ABSTRACT. The beneficial effects of early mycorrhizal inoculation with two arbuscular fungi, *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe and *Glomus intraradices* Schenck and Smith, were evaluated on Myrobalan 29 C (*Prunus cerasifera* × *Prunus munsoniana* Wight and Edr.) plum rootstock in soil infested or noninfested with the root lesion nematode *Pratylenchus vulnus* Allen and Jensen under microplot conditions. During this two year study, mycorrhizal colonization did not affect the number of nematodes per gram of root in plants infected with *P. vulnus*. In contrast, *P. vulnus* significantly decreased the percentage of mycorrhizal root colonization. Most elements were within sufficiency levels for plum by foliar analysis, although low P and deficient Fe and Cu levels were detected in *P. vulnus* treatments. Early mycorrhizal inoculation with *G. mosseae* favored plant growth after 20 months, but in soils infested by *P. vulnus*, only *G. intraradices* increased the tolerance of Myrobalan 29 C plum rootstock to damaging nematode levels by stimulating plant nutrition and vegetative growth.

Pratylenchus vulnus Allen and Jensen is considered the most important root-lesion nematode attacking fruit tree crops in Mediterranean environments (Corbett, 1974; Lamberti, 1981; Scotto La Massese, 1975). This nematode species is the primary causal agent of replant disease in many parts of the world (Nyczepir and Halbrecht, 1993) and is a major component of peach decline in warm climates (Day and Serr, 1953). In Spain, it is considered an important pest in nurseries and orchards where it has been found to be associated with tree decline (García de Otazo, 1992). Pathogenicity studies conducted in Spain and France indicate that this nematode is damaging to apple (*Malus silvestris* L.), pear (*Pyrus communis* L.), quince (*Cydonia oblonga* Miller), and several *Prunus* rootstocks including plums (Calvet et al., 1995; Fernández et al., 1992; Pinochet et al., 1993a; Scotto La Massese, 1989).

Plum rootstocks (*Prunus* spp.) are used in the Mediterranean region for both plum and peach cultivars. The plum rootstock Myrobalan 29 C, released in California in 1920 (Gregory Brothers Nursery Co., Brentwood, Calif.), has recently been introduced into Spain. It has shown important agronomic advantages over existing rootstocks and adapts well to heavy and calcareous soils (Felipe, 1989). This rootstock is in high demand by growers and is being used for establishing new orchards, as well as in replant situations in areas where root-lesion nematodes are common. In recent pathogenicity tests (Pinochet et al., 1996), Myrobalan 29 C was found to be susceptible to *P. vulnus*.

Arbuscular mycorrhizal (AM) fungi are obligate symbionts that increase nutrient uptake by plants, especially phosphorus and other minor elements (Gerdemann, 1968; Smith, 1987). Mycorrhizae

can also reduce damage caused by diseases (Perrin 1991) and nematode pests (Hussey and Roncadori, 1982; Franci, 1993). Interaction studies with AM fungi and *P. vulnus* on in vitro propagated apple (Pinochet et al., 1993 b), quince (Calvet et al., 1995), cherry (*Prunus mahaleb* L.) (Pinochet et al., 1995a), and pear (López et al., 1997) rootstocks have shown that mycorrhizal plants grew significantly more than noninoculated rootstocks in soil infested with the nematode.

The purpose of this investigation was to study the effects of the AM fungi *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe, and *Glomus intraradices* Schenck and Smith, on plant growth as compared with phosphorus fertilization, and on the damage caused by *P. vulnus* in micropropagated Myrobalan 29 C plum rootstock.

Materials and Methods

Micropropagated Myrobalan 29 C plum rootstock plants were supplied by Agromillora Catalana S.A., Sant Sadurní d' Anoya, Barcelona, Spain. Plants were received in small 100-cm³ pot trays containing sphagnum peat (TKS 1 Instant, Floragard GmbH, Germany) as a substrate. Plantlets were acclimatized in a greenhouse for 35 d and were transplanted into 24-L (40-cm-diameter) containers filled with a pasteurized (75 °C) sandy-loam textured soil (85% sand, 12% silt, 3% clay), pH 7.34, <2% organic matter and a cation exchange capacity of less than 10 meq/100 g soil. Phosphorus content of the soil at transplant fluctuated between 11 and 13 ppm, considered low but not deficient and a representative level of P status in soils dedicated to peach and plum production in Spain.

MICROPLOT EXPERIMENT. A microplot experiment lasting two years consisted of the following eight treatments: 1) control plants in low phosphorus soil (LP); 2) control plants in a soil with an optimum rate of phosphorus, hereafter designated as the high level (HP); 3) plants in low P soil inoculated with *P. vulnus* (Pv LP); 4) plants in the high P fertilization rate soil inoculated with *P. vulnus*

Received for publication 29 May 1997. Accepted for publication 19 Nov. 1997. We thank Agromillora Catalana, S.A., Sant Sadurní d' Anoya, Barcelona, for providing the plant material. This research was supported by the Instituto Nacional de Investigaciones Agrarias, INIA, Grant No. SC97-055. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

(Pv HP); 5) plants in low P soil inoculated with *G. intraradices* (Gi LP); 6) plants in low P soil inoculated with *G. mosseae* (Gm LP); 7) plants in low P soil with combined inoculations of *G. intraradices* and *P. vulnus* (Gi + Pv LP); and 8) plants in low P soil with combined inoculations of *G. mosseae* and *P. vulnus* (Gm + Pv LP).

During transplant to 24-L containers (March 1994), plants from mycorrhizal treatments were separately inoculated with 10 g of *G. intraradices* and *G. mosseae* soil inoculum. The inocula were rhizosphere soils from leek (*Allium porrum* L.) pot cultures, containing either 35 sporocarps of *G. mosseae* in 20 cm³ of soil, or heavily infected root fragments of *G. intraradices* resulting in 0.75 propagules/g measured by the most probable number technique (MPN) (Daniels and Skinner, 1982). Nonmycorrhizal plants received a mixed filtrate of soil inocula free of AM propagules.

Two months after mycorrhizal inoculation (May 1994), plants from the nematode treatments were inoculated with *P. vulnus* (simulating nursery-field exposure of symbiont and pathogen). The nematode population was isolated from rose (*Rosa multiflora* L.) in Cabrlis, Barcelona, Spain, cultured monoxenically on carrot disks (Moody et al., 1973) and identified to species level by the Commonwealth Institute of Parasitology, St. Albans, U.K. Inoculum was recovered from cultures by adding sterile water and collecting the nematodes with a pipette. The volume of the inoculant nematode suspension was adjusted to deliver 1000 individuals per plant (equivalent to 40 nematodes per l of soil) divided among eight holes located at 6- to 8-cm distance from the base of the stem. At inoculation time, plants were 17 ± 2 cm high and had 10 to 14 leaves. Containers were buried in the soil spaced at 1 m apart in a bucket microplot setup (Barker, 1985) with 54% shade under field conditions until the conclusion of the study (November 1995). Each treatment was replicated eight times in a completely randomized design. Plants were watered as needed and fertilized weekly with a modified Hoagland's (Hoagland and Arnon, 1950) nutrient solution low in phosphorus (0.10 g PO₄KH₂/L), except for plants with a higher P rate (optimum for plum), which received a double amount of phosphorus (0.20 g PO₄KH₂/L). During the course of the study, ambient temperature in the microplot field fluctuated between 3 °C (winter) and 34 °C (summer).

Plant growth parameters (fresh and dry top mass, shoot diameter and shoot length) were recorded at the end of the first season at 6 months after nematode inoculation (October 1994). The same parameters plus fresh root mass, nematode reproduction and AM colonization were assessed at 18 months after nematode inoculation (October 1995).

To determine the nematode population density, soil was separated from roots and placed in a large pan. Roots were washed to remove soil particles and the resulting suspension was added to the pan containing the soil. Nematodes in soil were recovered by differential sieving and sugar flotation (Jenkins, 1964) from an homogenized 250-cm³ subsample (Campubí et al., 1993). A small homogenized root sample (5 g root) was used to estimate the percentage of AM internal root colonization. Nematodes in roots were extracted by cutting the remaining root system (whole roots) into 1-cm-long pieces, and macerating it with water in a commercial blender at 14,500 g_n for 30 s given as three bursts of 10-s intervals. The nematode suspension was then concentrated using 150-, 74-, and 25-µm-pore sieves (100-, 200-, and 500-mesh, respectively). Root tissue and debris collected on the 150-µm-pore sieve were discarded.

For assessing mycorrhizal colonization, 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 (Giovannetti and Mosse, 1980). The size of the samples was sufficient to count at least 150 intersects per sample. From each *G. intraradices* inoculated plant, with or without *P. vulnus*, 10-cm-long segments were excised after clarifying and staining the root, mounted on millimetric slides and observed under a light microscope. The number of internal vesicles and spores formed by *G. intraradices* per mm of colonized root was counted based on size and external morphology of these structures.

Dry shoot mass, fresh root mass, shoot diameter, and shoot length values were analyzed by a multifactorial ANOVA. Levels of significance of main treatments and their interactions were calculated and means compared by using Tukey's test ($P \leq 0.05$).

Table 1. Growth of Myrobalan 29 C plum rootstock evaluated under microplot conditions inoculated with 1000 *Pratylenchus vulnus* per plant in combination with *Glomus intraradices* or *Glomus mosseae* 6 months (first season) and 18 months (second season) after nematode inoculation.

		First season (1994)			Second season (1995)			
Treatment ^a		Fresh shoot mass	Shoot diam	Shoot length	Fresh shoot mass	Shoot diam	Shoot length	Fresh root mass
		(g)	(mm)	(cm)	(g)	(mm)	(cm)	(g)
Mycorrhiza	Nematode							
LP	Control	16.6	6.9	97.6	109.8	9.58	476.8	61.4
HP		22.8	7.7	127.9	132.5	10.95	460.1	67.5
<i>G. mosseae</i> LP		18.0	6.6	92.3	167.9	11.21	559.0	82.6
<i>G. intraradices</i> LP		20.2	7.7	121.1	130.3	9.49	628.7	50.2
LP	<i>P. vulnus</i>	7.7	5.2	65.9	86.9	7.96	380.6	44.7
HP		17.1	6.5	114.1	100.8	9.40	417.5	60.8
<i>G. mosseae</i> LP		17.0	6.2	93.2	116.8	8.72	455.8	54.6
<i>G. intraradices</i> LP		14.2	6.2	94.9	137.4	9.60	577.0	57.3
LSD _{0.05}		6.81	1.07	27.9	37.75	1.35	116.46	16.39
Analysis of variance ^b								
Mycorrhiza (M)		*	*	***	*	NS	**	NS
Nematode (N)		**	***	**	**	***	**	***
M × N		NS	NS	NS	NS	*	NS	*

^aLP = low P fertilization (0.10 g PO₄KH₂/L), HP = high P fertilization (0.20 g PO₄KH₂/L).

^bData are the means of eight replications.

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 2. Reproduction of *Pratylenchus vulnus* and percentage of mycorrhizal root colonization by *Glomus mosseae* and *Glomus intraradices* in Myrobalan 29 C plum rootstock 20 months after inoculation with the AM fungi and 18 months after inoculation with 1000 nematodes per microplot.

Treatment ²	Final nematode population (soil and roots)	Nematodes per gram of root	Mycorrhizal colonization (%)
<i>P. vulnus</i> LP	193,040 a ^y	2,674 ab	---
<i>P. vulnus</i> HP	177,559 a	1,586 a	---
<i>G. mosseae</i> LP	---	---	68.5 b ^x
<i>P. vulnus</i> + <i>G. mosseae</i> LP	232,806 ab	2,700 b	54.6 a
<i>G. intraradices</i> LP	---	---	85.2 b
<i>P. vulnus</i> + <i>G. intraradices</i> LP	372,145 b	2,842 b	71.5 a

²LP = Low phosphorus fertilization (0.10 g PO₄KH₂/L); HP = High phosphorus fertilization (0.20 g PO₄KH₂/L).

^yData are means of eight replications. Actual data are presented, but data were log₁₀(x + 1) transformed for analysis. Means in the same columns followed by the same letter do not differ according to Tukey's multiple range test ($P \leq 0.05$).

^xPaired means (for each mycorrhizal fungus) in the same columns followed by the same letter do not differ according to Tukey's multiple range test ($P \leq 0.05$). Actual data are presented for percentage of AM colonization based on arcsin transformed values for analysis.

Final nematode populations and number of nematodes per gram of root in mycorrhizal and non-mycorrhizal plants were also analyzed by ANOVA, although data were log₁₀(x + 1) transformed before analysis. Data on percentage of mycorrhizal root colonization were transformed to arcsin for analysis. Means were also compared by Tukey's test ($P \leq 0.05$).

FOLIAR ANALYSIS. Composite leaf samples were collected for macro (N, P, K) and micro (Ca, Mg, Fe, Mn, Cu, Zn) element determination 75 d before destructive harvest. Samples from 30 to 35 whole midshoot leaves were taken from each plant in midsummer, avoiding senescent or necrotic tissue. Leaves were thoroughly washed with mild detergent, rinsed three times in distilled water and prepared for analysis. Leaf samples were then dehydrated in a temperature controlled fan ventilated oven at 70 °C ± 1 during 48 h, ground in a ball mill obtaining a minimum of 2 to 3 g dry matter sample required for element analysis. Leaf samples were then digested in wet acid (Jones et al., 1991) using nitric and perchloric acids. Analysis for all elements (except N) was made with a F586-587 Varian Liberty 220 inductively coupled plasma (ICP) emission spectrometer (Munter and Grande, 1981). Two readings were made per sample. Nitrogen content was determined according to Kjeldahl procedure (Rund, 1984). Data were analyzed by ANOVA and means were compared using Tukey's test ($P \leq 0.05$).

HISTOPATHOLOGY. Scanning electron microscope (SEM) observations were conducted for examining possible nematode-mycorrhiza interactions. Selected root pieces from active growing tissue were washed free of soil particles, fixed in FAA and dehydrated in alcohol. Cellular contents of root tissues were digested, desiccated to critical point in CO₂, mounted on aluminum stubs and sputtercoated with gold. The material was examined at accelerating potentials of 15 kV.

Results

Significant interactions exist between AM treatments and *P. vulnus* for fresh root mass and shoot diameter (Table 1). Plants growing in soil infested with *P. vulnus* had smaller root systems and shoot diameters than plants growing in noninfested soil, despite mycorrhizal inoculation.

Pratylenchus vulnus exerted a detrimental effect on Myrobalan 29 C rootstock growing in low P soil. During the first growing season, fresh shoot mass, shoot diameter and shoot length were

negatively affected, whereas at the end of the second growing season, the damage caused by the nematode could be recorded on shoot diameter, and fresh root mass.

When plants were fertilized with an optimum rate of P, only shoot diameter in both seasons was significantly lower in the presence of *P. vulnus*. The effect caused by mycorrhizal inoculation differed depending on the AM fungus used. In plants colonized by *G. mosseae*, the nematode caused no damage after the first six months of growth; however, a reduction in fresh shoot mass, shoot diameter and fresh root mass was recorded after 18 months of growth. In contrast, when plants were inoculated with *G. intraradices*, *P. vulnus* negatively affected the shoot diameter during the first season, whereas no measurable plant damage was detected on plant growth by the end of the second season.

Final nematode population density in roots and soil was higher ($P \leq 0.05$) in *P. vulnus* + *G. intraradices* LP treatment than in the *P. vulnus* LP and *P. vulnus* HP treatments. No differences occurred between the *P. vulnus* + *G. mosseae* LP and *P. vulnus* + *G. intraradices* LP inoculated plants (Table 2). *Pratylenchus vulnus* HP infected rootstocks had lower numbers of nematodes per gram of root than both mycorrhizal combinations with *P. vulnus*, but did not differ from *P. vulnus* LP treatment. The percentage of AM colonization in the roots fluctuated from 54.6% (lowest) to 85.2% (highest). *Glomus mosseae* LP presented a higher percentage of mycorrhizal root infection as compared with *G. mosseae* LP + *P. vulnus*. Similarly, nematode-free plants inoculated with *G. intraradices* showed higher mycorrhizal colonization than plants infected with both *G. intraradices* + *P. vulnus*. The numbers of vesicles and internal spores of *G. intraradices* were not affected by *P. vulnus*.

Most elements were within sufficiency levels for plum (Table 3), although a few element deficiencies were detected by foliar analysis (Jones et al., 1991). Nitrogen levels were adequate. However, Control LP and *G. intraradices* LP plants had higher N levels than both non-mycorrhizal + *P. vulnus* plants and *P. vulnus* + *G. intraradices* LP plants. Calcium levels were sufficient but the level was lower in Control HP and *P. vulnus* LP plants than in Control LP, *P. vulnus* + *G. mosseae* LP and *P. vulnus* + *G. intraradices* LP plants. Magnesium content was within sufficiency levels for all treatments, although lower in *G. mosseae* when compared to Control LP, *P. vulnus* + *G. mosseae* LP and *P. vulnus* + *G. intraradices* LP. Potassium was also sufficient in all treatments, but *G. intraradices* inoculated plants exhibited lower

Table 3. Mineral constituents of composite leaf samples from Myrobalan 29 C plum rootstock inoculated with *Pratylenchus vulnus*, *Glomus mosseae* and *Glomus intraradices* alone and in combination 16 months after inoculation with the nematode.

Treatment ^c	Mineral constituent								
	% dry mass					ppm			
	N	Ca	Mg	K	P	Fe	Mn	Cu	Zn
Control LP	3.19 c ^y	2.61 cd	0.42 c	2.55 ab	0.18 b	74 abc	224 bc	7.2 de	28 ab
Control HP	3.06 abc	2.03 a	0.37 ab	2.59 abc	0.17 ab	94 c	172 ab	5.2 bc	25 ab
<i>P. vulnus</i> LP	2.96 a	1.91 a	0.38 ab	2.81 cd	0.13 a	58 a	144 a	4.0 ab	23 a
<i>P. vulnus</i> + HP	2.91 a	2.26 abc	0.41 abc	2.67 bcd	0.18 b	76 abc	278 cd	3.0 a	29 ab
<i>G. mosseae</i> LP	2.99 ab	---	0.36 a	2.88 d	0.16 ab	79 abc	320 d	5.5 bcd	26 ab
<i>P. vulnus</i> + <i>G. mosseae</i> LP	3.05 abc	2.46 cd	0.42 c	2.77 bcd	0.19 b	91 bc	216 bc	6.5 cde	30 ab
<i>G. intraradices</i> LP	3.18 bc	2.18 ab	0.38 abc	2.48 a	0.19 b	---	---	10.2 f	31 b
<i>P. vulnus</i> + <i>G. intraradices</i> LP	2.92 a	2.72 d	0.41 bc	2.60 abc	0.17 ab	70 ab	251 c	8.0 e	29 ab
Low level ^x	1.7–2.39	1.0–1.49	0.2–0.29	1.0–1.5	0.09–0.13	60–99	20–39	5–6	15–19
Sufficiency level	2.4–3.0	1.5–3.0	0.3–0.8	1.6–3.0	0.14–0.25	100–250	40–160	7–16	20–50
High level	3.1–4.0	3.1–4.0	0.81–1.1	3.1–4.0	0.26–0.4	251–500	161–400	17–30	51–70

^aLP = low phosphorus fertilization (0.10 g PO₄KH₂/L); HP = high phosphorus fertilization (0.20 g PO₄KH₂/L).

^yData are means of eight replications. Means in the same columns followed by the same letter do not differ according to Tukey's multiple range test ($P \leq 0.05$).

^xInterpretative values for element content in plum by foliar analysis (Jones et al., 1991).

levels than both *P. vulnus* nonmycorrhizal plants and *G. mosseae* with and without the nematode. A low P content was found in *P. vulnus* LP treatment plants, the rest being within sufficiency levels. This treatment differed from Control LP, *P. vulnus* HP, *G. intraradices* LP, and *P. vulnus* + *G. mosseae* LP. Iron was insufficient in all treatments and deficient in *P. vulnus* LP plants. Control HP plants exhibited the highest Fe levels, above those of *P. vulnus* LP and *P. vulnus* + *G. intraradices* LP plants. Nearly all the treatments showed a high Mn content with the exception of *P. vulnus* LP (sufficient). *P. vulnus* LP had significantly lower levels of Mn than all other treatments except Control HP. Plants from both nematode non-mycorrhizal treatments were deficient in Cu and differed from most treatments. Plants from the remaining treatments exhibited varying levels of Cu leaf content. Copper was highest in *G. intraradices* LP plants. Zinc levels were within sufficiency levels for all treatments. *Pratylenchus vulnus* LP plants had lower Zn levels than plants inoculated with *G. intraradices* LP. In general, visual plant deficiency symptoms were observed for Fe and Cu in six *P. vulnus* LP and one *P. vulnus* HP microplots.

Light microscope and SEM observations of selected root fragments revealed extensive colonization of the nematode and of both endomycorrhizal fungi in the tissues of the cortical parenchyma. *Pratylenchus vulnus* was not detected in the conductive tissues. All life stages of the nematode were abundant, sometimes forming clusters of eggs, as well as typical cavities (collapse and dissolution of cell walls). Larval and adult forms normally aligned parallel to the stele. In combined inoculations, *P. vulnus* and both symbionts were found together colonizing the same spaces (Fig. 1A). Nematode migration within the roots was not restricted by mycorrhizae. *Glomus mosseae* colonization was strictly arbuscular. The production of vesicles and internal spores in plants colonized by *G. intraradices* was abundant irrespective of the nematode presence in the soil and the plants (Fig. 1B).

Discussion

Glomus mosseae and *G. intraradices* had no effect on nematode reproduction. This was clearly evident when Myrobalan 29 C rootstock inoculated with *P. vulnus* + *G. intraradices* LP showed

a significantly higher final nematode population density than non-mycorrhizal nematode inoculated plants. This was the result of a larger root system capable of supporting greater numbers of nematodes (Table 2). However, when the nematode root densities were expressed on a per gram root mass basis, both mycorrhizal treatments showed similar root densities (dilution of the root nematode population) when compared to the *P. vulnus* LP treatment. On the other hand, high P fertilization appears to have affected *P. vulnus* reproduction. High levels of P in soil have previously reduced buildup of *Radopholus similis* (Cobb) Thorne, in the roots of citrus seedlings (Smith and Kaplan, 1988).

In other interaction studies conducted with migratory endoparasitic species, nematode root densities were not affected by mycorrhizal infection, as reported with *R. similis* in citrus (O'Bannon and Nemec, 1979), and with *P. vulnus* in quince (Calvet et al., 1995), cherry (Pinochet et al., 1995a) and various plum rootstocks (Camprubí et al., 1993). This would indicate that both pathogen and symbiont are able to cohabit in the root cortical tissues and that the rootstock growth response can mainly be attributed to an increased capacity for nutrient uptake, and not to a direct effect of the mycorrhizae inhibiting nematode reproduction. This pattern of behaviour seems to be common for migratory endoparasitic nematodes in many perennial crops (Pinochet et al., 1996). However, reports in which mycorrhiza suppressed nematode reproduction have also been described for *G. intraradices* and *Radopholus citrophilus* Huettel, Dickson and Kaplan in rough lemon seedlings (Smith and Kaplan, 1988), and for *G. mosseae* and *P. vulnus* on Nemared peach rootstock (Pinochet et al., 1995b).

Pratylenchus vulnus significantly suppressed the root colonization by both AM fungi in the arbuscular phase in Myrobalan 29 C plum (Table 2). *Pratylenchus vulnus* is highly destructive on cortical parenchyma tissue (McElroy, 1972; Pinochet et al., 1993a), and is probably responsible for reducing the number of sites for fungal establishment and growth. In plants colonized by *G. intraradices*, the nematode did not reduce the production of vesicles and internal spores. Although the percentage of mycorrhizal root in plum was negatively affected by the nematode, *G. intraradices* colonization was still high (71.5%), and would seem sufficient to maintain a sustained production of reserve bodies (vesicles and internal spores). A high mycorrhizal dependency

Fig. 1. Roots of Myrobalan 29 C plum rootstock infected with *Pratylenchus vulnus* and the AM fungus *Glomus intraradices*. (A) Longitudinal section showing *P. vulnus* (nem) colonizing the cortical parenchyma of a feeder root. (B) Internal spores of *Glomus intraradices* (myc). Bar scale: A = 50 mm; B = 75 mm.

with significant increases in plant growth has also been reported for Marianna 2624 and Myrobalan 605 (*P. cerasifera* Ehr.) plum rootstocks inoculated with *G. mosseae* (Camprubí et al., 1993). Calvet et al. (1995) also reported that the percentage of root colonization, and especially the number of vesicles of *G. intraradices* per root fragment, was notoriously reduced in relation to nematode-free mycorrhizal BA-29 quince rootstock. The higher host capacity of quince to *P. vulnus* (higher nematode buildup in the roots), and thus more damage caused, would explain a higher level of mycorrhizal suppression and impaired production of reserve organelles (vesicles).

Nearly all element contents in leaf samples were within sufficiency levels for plum (Jones et al., 1991) with exception of P, Fe, and Cu. This was especially evident in plants from LP treatments without mycorrhizae which were low in P or deficient in Fe and Cu. Iron deficiency symptoms were visible in several trees of the LP treatment infested with *P. vulnus*. Mediterranean soils tend to be deficient in P and often are high in lime content (calcareous) with a tendency to immobilize oligoelements, mainly Fe (Vose, 1982). Breeding to solve the iron chlorosis problem on stone fruit crops is a priority (Socias i Company et al., 1995). The natural low availability of these elements compounded by the root damage caused by the root-lesion nematode feeding and migration within the roots can additionally impair nutrient uptake of these elements and originate a deficiency. In our study, none of the nematode infected plants with mycorrhizae

and fertilized with a low rate of P showed deficiency symptoms (levels were sufficient), underscoring the nutritional benefits of early mycorrhizal inoculation not only of P (Gerdemann, 1968), but also of Fe and Cu.

Our findings show that Myrobalan 29 C plum rootstock is susceptible to the root-lesion nematode *P. vulnus* in soils with low P availability. Both mycorrhizal treatments and HP fertilization conferred a certain degree of protection against *P. vulnus* after 6 months, but after 2 years of plant growth, only *G. intraradices* was still highly effective. *Pratylenchus vulnus* did not affect the development of plants colonized by *G. intraradices*, therefore the existence of the extramatrical fungal mycelium must be taken into account. This mycelial mass, although not measurable when the whole root system is considered, is responsible for the increased nutrient absorption in mycorrhizal plants.

Mycorrhizal plants colonized by *G. intraradices* tolerated the parasitism of this nematode. Root damage caused by *P. vulnus* probably did not affect growth development of Myrobalan 29 C plants under these circumstances. Therefore, early mycorrhizal inoculation with *G. intraradices* confers protection against *P. vulnus* on Myrobalan 29 C rootstock. The mycorrhizal fungus *G. mosseae*, very effective in nematode-free soil, was not a good endophyte for Myrobalan 29 C plants infected with *P. vulnus*. Our results indicate the importance in selecting the proper fungus for mycorrhizal mass-inoculation, especially when considering replant sites in mediterranean environments, where fruit-tree plantations have been established for a long time, and where the P level in these soils is normally very low. The utilization of mixed inocula containing a mixture of AM fungi is probably the best option in future inoculation trials for practical implementation in nursery production systems using micropropagated rootstocks.

Literature Cited

- Barker, K.R. 1985. Design of greenhouse and microplot experiments for evaluation of plant resistance to nematodes, p. 107–113. In: B.M. Zuckerman, W.F. Mai, and M.B. Harrison (eds.). Plant nematology laboratory manual. Univ. of Massachusetts Agricultural Experiment Station, Amherst.
- Calvet, C., J. Pinochet, A. Camprubí, and C. Fernández. 1995. Increased tolerance to the root-lesion nematode *Pratylenchus vulnus* in mycorrhizal micropropagated BA-29 quince rootstock. *Mycorrhiza* 5:253–258.
- Camprubí, A., J. Pinochet, C. Calvet, and V. Estaún. 1993. Effects of the root-lesion nematode *Pratylenchus vulnus* and the vesicular arbuscular mycorrhizal fungus *Glomus mosseae* on the growth of three plum rootstocks. *Plant Soil* 153:223–229.
- Corbett, D.C.M. 1974. *Pratylenchus vulnus*. C.I.H. descriptions of plant-parasitic nematodes. Set 3. No. 37. Commonwealth Inst. of Helminthology, St. Albans, Hertfordshire, England.
- Daniels, B.A. and H.B. Skinner. 1982. Methods for recovery and quantitative estimation of propagules from soil, p. 29–35. In: N.C. Schenck (ed.). Methods and principles of mycorrhizal research. Amer. Phytopathol. Soc., St. Paul, Minn.
- Day, L.H. and E.F. Serr. 1953. Comparative resistance of rootstocks of fruit and nut trees to attack by a root-lesion or meadow nematode. *Proc. Amer. Soc. Hort. Sci.* 57:150–154.
- Felipe, A.J. 1989. Patrones para frutales de pepita y hueso. Ediciones Técnicas Europeas, SA, Barcelona.
- Fernández, C., J. Pinochet, and R. Dolcet. 1992. Host-parasite relationship of *Pratylenchus vulnus* on apple and pear rootstocks. *Nematropica* 22:227–236.
- Francel, L.J. 1993. Interactions of nematodes with mycorrhizae and mycorrhizal fungi, p. 203–216. In: M.W. Khan. (ed.). Nematode interactions. Chapman and Hall, London.
- García de Otazo, L. 1992. La problemática de la replantación de frutales

- en las comarcas frutícolas de Lleida. *Fruticultura Profesional* 44:4–20.
- Gerdemann, J.W. 1968. Vesicular arbuscular mycorrhiza and plant growth. *Annu. Rev. Phytopathol.* 6:396–418.
- Giovanetti, M. and B. Mosse. 1980. An evaluation of techniques for measuring vesicular–arbuscular mycorrhizal infection in roots. *New Phytol.* 84:489–450.
- Hoagland, D. and D.I. Arnon. 1950 The water culture method for growing plants without soil. Univ. of California, Calif. Agr. Expt. Sta. Circ. 347.
- Hussey, R.S. and R.W. Roncadori. 1982. Vesicular–arbuscular mycorrhizae may limit nematode activity and improve plant growth. *Plant Dis.* 66:9–14.
- Jenkins, W.R. 1964. A rapid centrifugal flotation technique for separating nematodes from soil. *Plant Dis. Rpt.* 48:692.
- Jones, J.B., W. Benjamin, and H.A. Mills. 1991. Plant analysis handbook. I. Methods of plant analysis and interpretation. Micro–Macro Publishing, Athens, Ga.
- Koske, R.E. and J.H. Gemma. 1989. A modified procedure for staining roots to detect VA mycorrhizas. *Mycol. Res.* 92:86–505.
- Lamberti, F. 1981. Plant nematode problems in the Mediterranean region. *Helminthological Abstr. Ser. B. Plant Nematol.* 50:145–166.
- López, A., J. Pinochet, C. Fernández, C. Calvet, and A. Camprubí. 1997. Growth response of OHF-333 pear rootstock to arbuscular mycorrhizal fungi, phosphorus nutrition and *Pratylenchus vulnus* infection. *Fund. Appl. Nematol.* 20:87–93.
- McElroy, F.D. 1972. Nematodes of tree fruits and small fruits, p. 335–376. In: J.M. Webster (ed.). *Economic nematology*. Academic Press, London.
- Moody, E.H., B.F. Lownsbery, and J.M. Ahmed. 1973. Culture of the root-lesion nematode *Pratylenchus vulnus* on carrot disks. *J. Nematol.* 5:225–226.
- Munter, R.C. and R.A. Grande. 1981. Plant tissue and soil extract analysis by ICP–atomic emission spectrometry, p. 653–672. In: R.M. Barnes (ed.). *Developments in atomic plasma spectrochemical analysis*. Heyden & Son, Ltd., London.
- Nyczepir, A.P. and J.M. Halbrecht. 1993. Nematode pests of deciduous fruit and nut trees, p. 381–425. In: K. Evans, D.J. Trudgill, and J. M. Webster (eds.). *Plant parasitic nematodes in temperate agriculture*. CAB Intl., Wallingford, UK.
- O'Bannon, J.H. and S. Nemec. 1979. The response of *Citrus limon* seedlings to a symbiont, *Glomus etunicatus* and a pathogen, *Radopholus similis*. *J. Nematol.* 11:270–275.
- Perrin, R. 1991. Mycorrhizes et protection phytosanitaire, p. 93–130. In: D.G. Strullu (ed.). *Les mycorrhizes des arbres et plantes cultivées*. Technique et Documentation Lavoisier, Paris.
- Phillips, J.M. and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* 55:158–161.
- Pinochet, J., J. Marull, R. Rodríguez-Kábana, A. Felipe, and C. Fernández. 1993a. Pathogenicity of *Pratylenchus vulnus* on plum rootstocks. *Fund. Appl. Nematol.* 16:375–380.
- Pinochet, J., A. Camprubí, and C. Calvet. 1993b. Effects of the root-lesion nematode *Pratylenchus vulnus* and the mycorrhizal fungus *Glomus mosseae* on the growth of EMLA-26 apple rootstock. *Mycorrhiza* 4:79–83.
- Pinochet, J., C. Calvet, A. Camprubí, and C. Fernández. 1995a. Interaction between the root-lesion nematode *Pratylenchus vulnus* and the mycorrhizal association of *Glomus intraradices* and Santa Lucia 64 cherry rootstock. *Plant Soil* 170:323–329.
- Pinochet, J., C. Calvet, A. Camprubí, and C. Fernández. 1995b. Growth and nutritional response of Nemared peach rootstock infected with *Pratylenchus vulnus* and the mycorrhizal fungus *Glomus mosseae*. *Fund. Appl. Nematol.* 17:205–210.
- Pinochet, J., C. Fernández, E. Alcañiz, and A. Felipe. 1996. Damage by a lesion nematode, *Pratylenchus vulnus*, to *Prunus* rootstocks. *Plant Dis.* 80:754–757.
- Rund, R.C. 1984. Fertilizers, p. 8–37. In: S. Williams (ed.). *Official methods of analysis of the Association of Official Analytic Chemists*. 14th ed. AAOAC, Arlington, Va.
- Scotto La Massese, C. 1975. Tests d'hôtes de quelques porte-greffes et variétés fruitières à l'égard de *Pratylenchus vulnus* Allen et Jensen. *Comp. Rend. Acad. d'Agr. France.* 61:1088–1095.
- Scotto la Massese, C. 1989. Les problèmes posés par les nématodes phytophages à l'amandier, p. 33–38. In: A.J. Felipe and R. Socías (eds.). *Options méditerranéennes. Séminaire du GREMPA sur les porte-greffe de l'amandier*. CIHEAM, Zaragoza, España.
- Socías i Company, R., J. Gómez Aparisi, and A. Felipe. 1995. A genetical approach to iron chlorosis in deciduous fruit trees, p. 167–174. In: J. Abadía (ed.). *Iron nutrition in soils and plants*. Kluwer Academic Publishers, London.
- Smith, G.S. 1987. Interactions of nematodes with mycorrhizal fungi., p. 392–300. In: J.A. Veech and D.W. Dickson (eds.). *Vistas on nematology*. Soc. Nematol., Inc. Hyatsville, Md.
- Smith, G.S. and D.T. Kaplan. 1988. Influence of mycorrhizal fungus, phosphorus, and burrowing nematode interactions on growth of rough lemon citrus seedlings. *J. Nematol.* 20:539–544.
- Vose, P.B. 1982. Iron nutrition in plants: A world overview. *J. Plant Nutr.* 5:233–249.