Mycorrhiza (2001) 10:295–300

ORIGINAL PAPER

C. Calvet · J. Pinochet · A. Hernández-Dorrego
V. Estaún · A. Camprubí

Field microplot performance of the peach-almond hybrid GF-677 after inoculation with arbuscular mycorrhizal fungi in a replant soil infested with root-knot nematodes

Accepted: 6 December 2000

Abstract The effects of arbuscular mycorrhizae (AM) on the development and nutrition of the peach almond hybrid GF-677 rootstock in a replant soil heavily infested with *Meloidogyne javanica* were evaluated in field microplot conditions for two growing seasons. There was a significant beneficial effect of mycorrhizal inoculation on plant growth and nutrition in previously pasteurized replant soil. In natural replant soil, early inoculation with a mixed AM inoculum of *Glomus intraradices*, *Glomus mosseae* and *Glomus etunicatum* did not affect growth parameters. Whilst inoculation with these AM fungi led to suppression of root-knot nematode reproduction, natural mycorrhizal colonization of the replant soil with native AM fungi did not.

Keywords *Glomus* · *Meloidogyne javanica* · *Prunus* · Rootstock · Interaction

Introduction

Root-knot nematodes are regarded as economically important for stone fruit tree crops in warm Mediterranean climates (McElroy 1972; Lamberti 1981; Scotto La Massese 1989; Nyczepir 1991). There may be serious damage in early stages of plant development in the nursery or when rootstocks are transplanted into the field. In established orchards, they may cause delay in entering into production, lower yields, smaller fruit size, nutrient element deficiencies and a reduction in the longevity of the orchard (Nyczepir and Halbrendt 1993). New infestations occur with the introduction of infected plant material or through contaminated soil from nearby infested fields. Furrow irrigation contributes to the dispersion of these nematodes (McKenry 1988).

Orchards require renovation after loss of tree vigour, reduction in yields, changes in market demand for new fruit varieties and the introduction of new horticultural practices, such as the use of rootstocks that limit tree size and confer an invigorating or dwarfing effect (Felipe 1989). From the pathological standpoint, poor orchard development in replanted sites is common, and is expressed as failure of tree establishment, suppressed growth and shortened productive life. Replant problems are complex and are the result of several factors associated with each site (Mai and Abawi 1981). In southern Spain, root-knot nematodes are often considered as primary agents causing replant problems in warm sandy soils. Additional factors contributing to replant problems are poor soil structure, soil-borne pathogenic fungi and bacteria, low soil fertility and inadequate agronomic practices (García de Otazo 1992).

Root-knot nematodes cause extensive galling and the destruction of the root system, which results in loss of vigour and yield in both young and mature peach trees. Although root-knot nematode resistance is available in commercial *Prunus* rootstocks, the peach-almond hybrid GF-677 [*Prunus persica* Batch × *P. dulcis* (Mill.) Webb] is highly susceptible (Fernández et al. 1994). Nevertheless, this hybrid adapts well to dry land, calcareous soils and low fertility, conditions typical of Mediterranean environments, and is still widely used in replant situations. The extensive use of GF-677 has resulted in increasing replant problems caused by root-knot nematodes in the south and east of Spain (Tarragona, Valencia, Andalucia and Extremadura). Failure during establishment and poor growth after the first 2–3 years are common in replant situations. GF-677 established at new sites normally reaches high nematode densities after a few years in well-drained, coarse-textured soils. The renewal of plantations again with GF-677 following pullout results in further increases in specific replant problems caused by these pests.

C. Calvet (✉) · A. Hernández-Dorrego · V. Estaún · A. Camprubí
Departament de Protecció Vegetal,
Institut de Recerca i Tecnología Agroalimentàries, IRTA,
Ctra. de Cabrils s/n, 08348 Cabrils, Barcelona, Spain
e-mail: cinta.calvet@irta.es
Fax: +34-93-7533954

J. Pinochet
Agromillora Catalana SA, El Rebato s/n, 08739 T. M. Subirats,
Barcelona, Spain

Arbuscular mycorrhizal fungi (AMF) have been shown to promote plant growth by enhancing nutrition (Smith and Read 1997), by modifying root morphology (Berta et al. 1995) and by conferring protection against soil-borne pathogens (Hussey and Roncadori 1982; Smith 1987; Hooker et al. 1994; Azcón-Aguilar and Barea 1996). The beneficial effects of early mycorrhizal colonization of peach seedlings roots was first documented by Gilmore (1971), but information on growth responses of peach rootstocks in nematode-infested soils is limited and discrepant (Strobel et al. 1982; Pinochet et al. 1996).

The purpose of this present study was to determine the growth performance and the nutritional response under field microplot conditions of mycorrhizal GF-677 rootstocks established in a typical replant sandy soil from southern Spain heavily infested with the nematode *Meloidogyne javanica* (Treub) Chitwood.

Materials and methods

Replant soil

A replant soil (RS soil) infested with *M. javanica* was obtained from a peach orchard in San José de la Rinconada, Seville, Spain. The orchard was planted originally with GF-677 hybrid rootstock. Five-year-old plants were pulled out because of the overall damage caused by the root-knot nematodes (10% mortality per year and unproductiveness). The properties of this soil were: sandy loam (34% sand, 38% loam, 28% clay), pH 8.4, 6.9% active lime, 0.6% organic matter, conductivity 0.27 dS/m and a cation exchange capacity below 10 meq/100 g soil. The most significant plant-parasitic nematode present was *M. javanica* at a field density of 4,960 nematodes per litre of soil measured 3 weeks after pull-out in April 1997. Other nematodes present at lower densities were: *Paratylenchus neglectus* (Rensch) Filipjev and Schuurmans Stekhoven, *Paratylenchus* sp., *Trichodorus* sp., *Hemicriconemoides mangiferae* Siddiqi, and *Helicotylenchus erithrinae* (Zimmerman) Golden. These are thought to have no economic importance for peach and were probably associated with adjacent weeds.

Plant material

Micropropagated GF-677 plantlets were received from Agromillora Catalana SA, Sant Sadurní d' Anoia, Barcelona, Spain. Plantlets were transferred from agar to 50-ml minipots filled with a peat (Floratorf, Floraguard GmbH, Germany) and perlite (Iberperlita, Stavik SA, Huesca, Spain) substrate mixture and placed in a high-humidity chamber (90–95% RH) at 25°C for 24 days.

AM inoculum and plant inoculation

Three fungi were used in a mixed AM soil inoculum: *Glomus intraradices* Schenck and Smith, *Glomus mosseae* (Nicolson and Gerdemann) Gerd. and Trappe and *Glomus etunicatum* Becker and Gerdemann. The isolates of *G. intraradices* and *G. mosseae* are registered in the European Bank of Glomales as BEG ID 72 and BEG ID 116, respectively. *G. intraradices* was isolated from a citrus orchard in Tarragona, Spain. The *G. mosseae* isolate was obtained in 1981 from Rothamsted Experimental Station, UK and *G. etunicatum* was isolated from *Trifolium repens* L. growing in a high pH sandy soil in Cabrils, Barcelona. The three isolates were grown separately in leek (*Allium porrum* L.) pot cultures on autoclaved sandy soil in a greenhouse under controlled conditions. Rhizosphere soils containing abundant propagules of each AM fungus were mixed thoroughly and the soil mixture used as inoculum. The resulting concentration of mycorrhizal propagules was approximately 300 spores of *G. intraradices*, 15 sporocarps of *G. mosseae* and 150 spores of *G. etunicatum* in 10 g of soil. Spore numbers were recorded after wet sieving (Daniels and Skinner 1982), including root shredding in a blender for *G. intraradices*-infected root fragments. Plantlets with uniform growth were transplanted to 300-ml containers filled with a pasteurized sandy soil-quartz sand-sphagnum peat 4:3:1 (v/v) substrate mixture. Half of the plants were inoculated with 10 g per plant of the AM inoculum at transplant. The pots were kept in the greenhouse for 45 days, after which the plants were transplanted to replant soil, pasteurized or non-pasteurized (i.e. containing the original microbiota) in microplot conditions.

Microplot experiments

Two field microplot experiments were established with four identical treatments lasting one or two growing seasons: 1) RS-control, 2) RS soil inoculated with AMF (*G. mosseae* + *G. intraradices* + *G. etunicatum*) (RS-AMF), 3) pasteurized RS soil (PRS-control), 4) PRS inoculated with AMF (PRS-AMF). The experiments were established in April 1997. GF-677 plants were transplanted individually into 10-l bucket microplots (Barker 1985) and arranged in a completely randomized design with 20 replicates per treatment. Eight plants per treatment were used for the experiment lasting for one growing season (7 months from April to November 1997) and twelve plants per treatment were used in the experiment lasting for two growing seasons (18 months from April 1997 to October 1998). Plants were irrigated as needed and fertilized with 10 g per plant of a slow-release fertilizer (Osmocote Plus,15–10–12 + micronutrients; Sierra Grace España, SA, Tarragona) in May 1997 and May 1998. From May to September, plants were supplemented weekly with 100 ml of Hoagland's nutrient solution (Hoagland and Arnon 1950) lacking P alternating with full-strength. Microplots were established 80 cm apart in a shaded area (54%) in the field.

Plant growth and nematode density

Plant growth (shoot and root weights, plant height, stem diameter) and nematode density were assessed at the end of the first and second growing seasons. Stem diameter measurements were made 3 cm above the soil line. The percentage of galled root system (Barker 1985), the final nematode population density in roots and soil and the number of nematodes per gram fresh root weight were assessed at the end of each experiment. Soil recovered from each microplot was separated from roots and placed in a large pan with water. Roots were washed in a second pan to remove soil particles and the resulting suspension was added to the pan containing the soil and stirred thoroughly. Nematodes in soil were extracted from a 250-ml aliquot of the slurry by differential sieving through 100-, 200- and 400-meshes (150, 74 and 38 µm, respectively) and sugar flotation (Jenkins 1964). Nematodes in roots were extracted from whole root systems cut into 1-cm-long pieces and macerated in water in a commercial blender at 14,500 rpm for 30 s given at 10-s intervals. The nematode suspension was concentrated with 100-, 200- and 500-mesh sieves (150, 74 and 25 µm, respectively). Root tissue and debris collected on the 150-µm sieve were discarded. Nematodes were recovered in the remaining sample by sugar flotation (Jenkins 1964).

AM colonization

For assessing AM colonization, root samples were stained with 0.05% trypan blue in lactic acid after Phillips and Hayman (1970) as modified by Koske and Gemma (1989). The percentage of root colonization was determined using the grid-line intersect method (Giovanetti and Mosse 1980). Samples were large enough to count at least 150 intersects per sample.

Table 1 Plant growth response and mycorrhizal colonization of GF-677 plants inoculated or not with three arbuscular mycorrhizal fungi (AMF): *Glomus intraradices*, *Glomus etunicatum* and *Glomus mosseae* in a replant soil (RS) infested with *Meloidogyne javanica* and in pasteurized replant soil (PRS). Parameters were measured at the end of the first growing season 7 months after transplant to microplots (means of eight replicates) and at final harvest 18 months after transplant to microplots (means of twelve replicates). Different letters in the same column indicate significant differences between treatments at the end of the first growing season or at final harvest

Treatment	Time after transplant (months)	Stem diameter (mm)	Plant height (cm)	Shoot fresh wt. (g)	Root fresh wt. (g)	Shoot dry wt. (g)	Mycorrhizal colonization (%)
RS-control	7	6.08 a	142.92 a	22.51 a	39.41 ab	10.33 a	90.98 a
RS-AMF		6.19 a	96.15 a	17.71 a	38.41 ab	8.20 a	92.02 a
PRS-control		6.06 a	75.37 a	19.78 a	27.05 a	9.24 a	
PRS-AMF		8.06 b	259.69 b	54.73 b	44.25 b	26.26 b	87.87 a
RS-control	18	9.58 a	737.66 ab	157.49 a	105.12 a	69.80 a	85.12 a
RS-AMF		9.72 a	770.78 b	160.99 a	104.52 a	67.00 a	81.02 a
PRS-control		10.88 a	605.43 a	169.84 ab	89.84 a	82.04 b	
PRS-AMF		11.30 a	728.16 ab	190.80 b	87.73 a	86.86 b	85.68 a

Table 2 Reproduction of *Meloidogyne javanica* in GF-677 plants inoculated or not with three AMF: *Glomus intraradices*, *G. etunicatum* and *G. mosseae* in a naturally infested replant soil (RS). Parameters were measured at the end of the first growing season (means of eight replicates) or at final harvest (means of twelve replicates). Different letters in the same column indicate significant differences between treatments at the end of the first growing season or at final harvest

Treatment	Time after transplant (months)	Root galling (%)	Final nematode population (root)	Number of nematodes (per g root)	Total nematodes (soil+root)
RS-control	7	12.63 b	128857 b	3280.64 b	133829 b
RS-AMF		4.88 a	52945 a	1323.25 a	55414 a
RS-control	18	65.75 a	115015 b	1088.64 b	120036 b
RS-AMF		54.91 a	74543 a	679.17 a	79706 a

Mineral composition

The concentrations in leaves of N, P, K, Ca, Mg, Fe, Cu, Mn and Zn were determined for each growing season according to procedures established for peach (Jones et al. 1991). Boron (B) was determined only in the second year. Whole midshoot leaves (35–40 from each tree) were collected in midsummer avoiding senescent tissue. Leaves were washed in mild detergent, rinsed thoroughly in distilled water and dehydrated in a fan-ventilated oven (70°C). The dry leaves were ground in a ball mill and a minimum of 1.8–2.2 g of dry matter used for element analysis. Analysis of all elements except N was carried out in a F586–587 Varian Liberty 220 inductively coupled plasma (ICP) emission spectrometer (Munter and Grande 1981). Nitrogen content was determined by the Kjeldahl procedure (Rund 1984).

Statistical analysis

Data recorded for plant growth, mycorrhizal root colonization and element content in leaves were analysed by ANOVA ($P \leq 0.05$) and mean values compared with Tukey's multiple range test. Six and eight replicates of foliar tissue were used to determine mineral composition in the first and second growing seasons, respectively. Nematode reproduction data were obtained from only two treatments, as no nematodes were present in PRS; hence means were analysed by Student's *t* test. Data on the final nematode population and number of nematodes per gram of root were $\log_{10}(x+1)$ transformed for analysis. Data on the percentage of galled root system and mycorrhizal colonization in roots were arcsin transformed ($\sqrt{x/100}$) for analysis.

Results

In the first growing season, mycorrhizal inoculation caused no significant increase in plant growth (RS-AMF versus RS-control). However, when plants were transplanted to pasteurized soil, artificially inoculated plants (PRS-AMF) showed significant increases in stem diameter, plant height, fresh and dry shoot weights. Fresh root weights of PRS-AMF plants were also higher than those of PRS-control plants, but did not differ from those of plants from either RS treatment (Table 1).

Differences in plant growth between treatments tended to disappear for most parameters in the second growing season (Table 1). RS-AMF plants were taller than those from the PRS-control treatment. PRS-AMF plants had a higher shoot fresh weight than plants from either RS treatment. Dry shoot weights of plants from both PRS treatments were also higher, despite AMF artificial inoculation at transplant.

GF-677 plants grown in the replant soil in both experiments were all highly mycorrhizal. This indicates the presence of infective native AMF propagules in the natural replant soil. The percentage of mycorrhizal colonization of GF-677 roots achieved by native fungi was as high (ca. 80%) 18 months after the transplant as that resulting from the introduced inoculum.

The percentage of AM colonization was very high in all cases (ca. 90% the first year and ca. 80% in the sec-

Table 3 Mineral composition of dried foliar tissue of GF-677 plants inoculated or not with three AMF: *Glomus intraradices, G. etunicatum* and *G. mosseae*, and growing in a replant soil (RS) naturally infested with *Meloidogyne javanica* or in pasteurized replant soil (PRS) The composition was measured during midsummer of the first (means of six replications) and second (means of eight replicates) growing seasons. Different letters in the same column indicate significant differences between treatments at the end of the first or second growing season

Treatment	Season	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Cu (ppm)	Mn (ppm)	Zn (ppm)	B (ppm)
RS-control	1st	2.54 b	0.19 b	1.24 b	1.61 c	0.43 c	207.70 a	4.93 b	60.79 a	17.26 a	
RS-AMF		2.45 ab	0.18 b	1.26 b	1.37 bc	0.40 bc	210.04 a	4.82 b	61.40 a	21.72 ab	
PRS-control		2.09 a	0.11 a	0.94 a	0.91 a	0.27 a	196.50 a	2.67 a	49.71 a	17.03 a	
PRS-AMF		2.21 ab	0.21 b	1.27 b	1.18 b	0.35 b	186.32 a	7.08 c	68.78 a	27.01 b	
RS-control	2nd	3.68 a	0.20 a	2.35 a	1.16 a	0.44 a	63.50 a	7.00 a	51.00 a	35.25 a	27.00 a
RS-AMF		3.65 a	0.19 a	2.46 a	1.59 b	0.51 b	73.25 a	7.25 a	63.25ab	47.00 b	33.50 b
PRS-control		3.65 a	0.21 a	2.22 a	1.63 b	0.48 ab	67.75 a	7.50 a	57.50 a	35.25 a	33.25 b
PRS-AMF		3.45 a	0.20 a	2.33 a	1.71 b	0.49 b	67.25 a	7.00 a	66.25 b	37.00 a	32.00 b

ond year). In PRS treatments, replant soil was pasteurized before filling the microplot buckets in order to simulate experimentally a fairly common situation in nurseries and orchards, i.e. the transplant of rootstocks to disinfected soils. When plants were inoculated with the mixed inoculum before transplant to the pasteurized soil, a positive effect on plant growth due to mycorrhizae was observed (Table 1).

Root galling was low in the first growing season (Table 2); RS-control plants had a threefold higher percentage of galling than mycorrhizal plants. Nematode proliferation was significantly lower in RS-AMF than in RS-control microplots. After the second growing season, root galling was high and no differences were detected between treatments (Table 2). Nematode proliferation remained significantly lower than controls in plants inoculated with the AM mixed inoculum at transplant (RS-AMF microplots).

At harvest, high nematode densities in roots were recorded in GF-677 plants not inoculated artificially with AM fungi (Table 2), although all plants growing in replant soil had been colonized naturally by native AM fungi propagules. Nematodes had no negative or suppressive effects on mycorrhizal colonization. In all cases, a high level of mycorrhizal colonization was recorded despite soil infestation by the pathogen (Table 1).

At the end of the first growing season, macro- and micronutrient levels (P, K, Ca, Mg, Cu) in leaves were lower for plants from the PRS-control treatment than from the other three treatments (Table 3). RS-control plants had higher levels of N than PRS-control plants, as well as higher levels of Ca and Mg than plants from both PRS treatments. Cu and Zn contents were highest in the PRS-AMF plants. One year later, the artificially introduced mycorrhizal fungi had enhanced Ca, Mg, Zn and B uptake in RS-AMF, especially relative to the RS-control treatment. Inoculated plants had higher Zn foliar contents than naturally mycorrhizal plants.

The leaf element content increased in the second year, except in the case of Fe, which was considerably reduced (up to threefold) but was not deficient. This was probably due to high absorption compounded by sequestration due to the calcareous nature and high pH of the soil, which is known to result in iron chlorosis. No visual deficiency symptoms were detected throughout the study. Mn was significantly higher in PRS-AMF plants than in non-inoculated RS or PRS plants. Regardless of treatment, most elements were at sufficiency levels for peach (Jones et al. 1991). Only Cu was deficient and low levels were recorded for N, P, K, Ca, Zn and Mg in the PRS-control plants.

Discussion

At the end of the first growing season, treatments with pasteurized replant soil into which a mix of three different AM fungi was inoculated indicated a stimulatory effect of mycorrhizae on plant performance (Table 1). In a previous study by Estaún et al. (1994), growth of GF-677 in different potting substrates was also stimulated by these isolates of *G. intraradices* and *G. mosseae*. Early artificial inoculation of rootstocks with selected AM fungi before transplantation to a fumigated or disinfected soil can be expected to be beneficial for plant development. In natural replant soil, native fungi colonized the roots of GF-677 plants and no differences were detected between inoculated and noninoculated plants for all growth parameters measured. Furthermore. no differences were detected between the mycorrhizal RS treatments and the nonmycorrhizal pasteurized treatments, despite the presence of pathogenic nematodes in RS treatments. Root galling caused by the nematode was low, but was significant in mycorrhizal plants artificially inoculated and established in replant soil.

At harvest after the second growing season, galling was high in all plants, indicating that mycorrhizal colonization does not decrease galling during long-term growth. Despite the similar level of root galling found between treatments (65.7% and 54.9%, respectively), there was a significant difference in the final number of nematodes within the root tissues, especially when expressed per gram root tissue (Table 2). This is considered to be the most reliable parameter for evaluating the pathogen in-

fection of roots. The lower level of nematode parasitism in the roots of plants colonized by AM fungi before transplant to the replant soil (60.8% and 38.6% lower at the end of the first and second growing seasons, respectively) is encouraging from the nematode control point of view, as it clearly indicates a suppressive effect of some AMF on *M. javanica*. The symbiont appears to compete advantageously for food resources and space once well established. The mechanisms of suppression are unknown, but would appear to be associated with physiological changes making root tissue an unfavourable food source for the root-knot nematode species. On the other hand, the nematode had no effect on mycorrhizal development.

There is little information about interactions between root-knot nematodes and mycorrhizae in peach or other perennial fruit crops. Strobel et al. (1982) reported a suppressive effect of *Gigaspora margarita* on the reproduction of *Meloidogyne incognita* only in tests where the fungus improved peach growth. In that same study, *Glomus etunicatum* showed no effect on nematode reproduction. Early mycorrhizal inoculation of banana plants with *Glomus mosseae* suppressed both root galling and *M. incognita* build-up in the roots (Jaizme-Vega et al. 1997). Other authors have also reported a decrease in root-knot nematode populations due to mycorrhizal infection of roots of important annual crop species, e.g. onion (*Allium cepa* L.), soybean (*Glycine maxima* L.. Merr.), cotton (*Gossypium herbaceum* L.) and tomato (*Lycopersicon esculentum* Mill.) (Hussey and Roncadori 1982).

When uninoculated GF-677 plants became naturally mycorrhizal in the replant soil, native AMF and nematodes apparently had the same opportunity in time to penetrate and compete in the root tissue. Thus, no suppressive effect on nematode infection was detected, despite the high level of root colonization by the native AMF. These results show the practical importance of early artificial inoculation with selected, efficient AMF able to protect the rootstock against nematodes when orchards are established in replant soils. Mycorrhizal inoculation of plants in the nursery before transplant allows the fungi sufficient time to establish an efficient symbiosis in the roots prior to pathogen exposure.

The only treatment lacking mycorrhiza (PSR-control) produced lower levels of P, K, Ca, Mg, and Cu than other treatments in the first season. Furthermore, GF-677 plants colonized by the mixed inoculum (PRS-AMF and RS-AMF) showed a significant increase in uptake of Ca, Mg, Mn, Zn and B in the second growing season relative to the controls. Our results are consistent with the foliar increases in P, Cu and Zn reported by Strobel et al. (1982) for peach trees grown in *M. incognita*-infested soil and inoculated with *Gigaspora margarita* and *Glomus etunicatum*. Similar results were obtained by Gilmore (1971) with mycorrhizal peach seedlings: foliar Zn levels were 2–3 times higher than those of control seedlings, preventing the appearance of Zn-deficiency symptoms. AMF have also been found to be equally or more effective in overcoming soil-fumigation nutrient deficiency effects in peach nursery seedlings than the standard nursery practice of side dressing P and Zn at planting time (La Rue et al. 1975).

Increases in nutrient uptake by inoculated mycorrhizal plants have been recorded for other fruit tree species, such as quince BA-29 rootstock inoculated early with *Glomus intraradices* (Calvet et al. 1995), cherry Santa Lucia 64 rootstock with *G. intraradices* (Pinochet et al. 1995) and the Myrobalan 29C plum rootstock inoculated with *G. intraradices* and *G. mosseae* (Pinochet et al. 1998), all established in lesion nematode (*Pratylenchus vulnus*) infested soil. Most of the results clearly indicate increases in the P nutrition of mycorrhizal plants and in the uptake of low diffusion elements like Fe, Mn, Cu and Zn. This increased the availability of these elements to trees in the initial stages of active vegetative growth.

Our findings infer that serial inoculation of plants with selected AMF which confer tolerance by stimulating growth and nutrition and suppressing root-knot nematode reproduction in the roots should be considered as an alternative to costly soil desinfestation in replant situations when rootstocks susceptible to root-knot nematodes are being used. This is the case for GF-677, the rootstock most widely used for peach in Spain currently and the main choice for replant situations. The results obtained in the first 2 years of tree growth are encouraging but should be considered incomplete. The significance of early mycorrhizal colonization of GF-677 in root-knot nematode infested soil for tree survival after the first years of establishment and for fruit production still remains to be determined in long-term field testing.

Acknowledgement This research was supported by the Instituto Nacional de Investigaciones Agrarias, INIA, Grant No. SC97–055.

References

Azcón-Aguilar C, Barea JM (1996) Arbuscular mycorrhizas and biological control of soil-borne plant pathogens. An overview of the mechanisms involved. Mycorrhiza 6:457–464

Barker KR (1985) Design of greenhouse and microplot experiments for evaluation of plant resistance to nematodes. In: Zuckerman BM, Mai WF, Harrison MH (eds) Plant nematology laboratory manual. University of Massachusetts Agricultural Experiment Station, Amherst, Mass, pp 103–113

Berta G, Trotta A, Fusconi A, Hooker JE, Munro M, Atkinson D, Giovannetti M, Morini S, Fortuna P, Tisserant B, Gianinazzi-Pearson V, Gianinazzi S (1995) Arbuscular mycorrhizal induced changes to plant growth and root system morphology in *Prunus cerasifera*. Tree Physiol 15:281–293

Calvet C, Pinochet J, Camprubí A, Fernández C (1995) Increased tolerance to the root-lesion nematode *Pratylenchus vulnus* in mycorrhizal micropropagated BA-29 quince rootstock. Mycorrhiza 5:253–258

Daniels BA, Skinner HB (1982) Methods for recovery and quantitative estimation of propagules from soil. In: Schenck NC (ed) Methods and principles of mycorrhizal research. American Phytopathological Society, St. Paul, Minn, pp 29–35

Estaún V, Calvet C, Camprubí A (1994) Arbuscular mycorrhizae and growth enhancement of micropropagated *Prunus* rootstocks in different soil-less potting mixes. Agric Sci Finl 3:263–268

Felipe AJ (ed) (1989) Patrones para frutales de pepita y hueso. Ediciones Técnicas Europeas, Barcelona

Fernández C, Pinochet J, Esmenjaud D, Salesses G, Felipe A (1994) Resistance among new *Prunus* rootstocks and selections to root-knot nematodes from Spain and France. HortScience 29:1064–1067

García de Otazo L (1992) La problemática de la replantación de frutales en las comarcas frutícolas de Lleida. Frut Prof 44:4–20

Gilmore AE (1971) The influence of endotrophic mycorrhizae on the growth of peach seedlings. J Am Soc Hortic Sci 96:35–38

Giovanetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol 84:489–450

Hoagland D, Arnon DI (1950) The water culture method for growing plants without soil. Calif Agric Exp St Circ 347

Hooker JE, Jaizme-Vega M, Atkinson D (1994) Biocontrol of plant pathogens using arbuscular mycorrhizal fungi. In: Gianinazzi S, Schüepp H (eds) Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems. Birkhaüser, Basel, pp 191–200

Hussey RS, Roncadori RW (1982) Vesicular-arbuscular mycorrhizae may limit nematode activity and improve plant growth. Plant Dis 66:9–14

Jaizme-Vega MC, Tenoury P, Pinochet J, Jaumot M (1997) Interactions between the root-knot nematode *Meloidogyne incognita* and *Glomus mosseae* in banana. Plant Soil 196:27–35

Jenkins WR (1964) A rapid centrifugal flotation technique for separating nematodes from soil. Plant Dis Rep 48:692

Jones JB, Benjamin W, Mills HA (1991) Plant analysis handbook. 1. Methods of plant analysis and interpretation. Micro-Macro, Athens, Ga

Koske RE, Gemma JH (1989) A modified procedure for staining roots to detect VA mycorrhizas. Mycol Res 92:6–505

La Rue JH, McClellan WD, Peacock WL (1975) Mycorrhizal fungi and peach nursery nutrition. Calif Agric 5:6–7

Lamberti F (1981) Plant nematode problems in the Mediterranean region. Commonwealth Institute of Helminthology, St. Albans, UK, Helminthological Abstracts, Ser B, Plant Nematol 50: 145–166

Mai WF, Abawi GS (1981) Controlling replant disease on pome and stone fruit in Northeastern United States by preplant fumigation. Plant Dis 65:859–864

McElroy FD (1972) Nematodes of tree fruits and small fruits. In: Webster JM (ed) Economic nematology. Academic, London, pp 335–376

McKenry MV (1988) Nematodes. In: La Rue JH, Scott Johnson R (eds) Peaches, plums and nectarines. Growing and handling for fresh market. Cooperative Extension, University of California, Division of Agriculture and Natural Resources, Publication 3331, pp 139–147

Munter RC, Grande RA (1981) Plant tissue and soil extract analysis by ICP-atomic emission spectrometry. In: Barnes RM (ed) Developments in atomic plasma spectrochemical analysis. Heyden, London, pp 653–672

Nyczepir AP (1991) Nematode management strategies in stone fruits in the United States. J Nematol 23:334–341

Nyczepir AP, Halbrendt JM (1993) Nematode pests of deciduous fruit and nut trees. In: Evans K, Trudgill DL, Webster JM (eds) Plant parasitic nematodes in temperate agriculture. CAB International, Wallingford, UK, pp 381–425

Phillips JM, Hayman DS (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, Calvet C, Camprubí A, Fernández C (1995) 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, Calvet C, Camprubí A, Fernández C (1996) Interactions between migratory endoparasitic nematodes and arbuscular mycorrhizal fungi in perennial crops: a review. Plant Soil 185:183–190

Pinochet J, Camprubí A, Calvet C, Fernández C, Rodríguez-Kábana R (1998) Inducing tolerance to the root-lesion nematode *Pratylenchus vulnus* by early mycorrhizal inoculation of micropropagated Myrobalan 29C plum rootstock. J Am Soc Hortic Sci 123:342–347

Rund RC (1984) Fertilizers. In: Williams S (ed) Official methods of analysis of the Association of Official Analytical Chemists, 14th edn. AAOAC, Arlington, Va, pp 8–37

Scotto La Massese C (1989) Les problèmes posés par les nématodes phytophages à l'amandier. In: Felipe AJ, Socías R (eds) Options méditerranéennes. Séminaire du GREMPA sur les Porte-Greffe de l'Amandier. CIHEAM, Saragossa, Spain, pp 33–38

Smith GS (1987) Interactions of nematodes with mycorrhizal fungi. In: Veech JA, Dickson DW (eds) Vistas on nematology. Society of Nematologists, Hyatsville, Md, pp 392–400

Smith SE, Read DJ (1997) Mineral nutrition, heavy metal accumulation and water relations of VA mycorrhizal plants. In: Smith SE, Read DJ (eds) Mycorrhizal symbiosis, 2nd edn. Academic, London, pp 126–160

Strobel NE, Hussey RH, Roncadori RW (1982) Interactions of vesicular-arbuscular mycorrhizal fungi, *Meloidogyne incognita*, and soil fertility on peach. Phytopathology 72:690–694