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Increased tolerance to the root-lesion nematode *Pratylenchus vulnus* in mycorrhizal micropropagated BA-29 quince rootstock

Abstract The interaction between *Glomus intraradices* and the root-lesion nematode *Pratylenchus vulnus* was studied on micropropagated BA-29 quince rootstock during one growing season. Inoculation with *G. intraradices* significantly increased growth of plants in low P soil and was more effective than P fertilization at increasing top-plant development. In the presence of the nematode, mycorrhizal plants achieved higher values in all growth parameters measured. *P. vulnus* caused a significant decrease in the percentage of root length colonized by *G. intraradices* and fewer internal vesicles were formed within the host roots. Enhanced root mass production accounted for the twofold increase in final nematode population recovered from plants with combined inoculations of pathogen and symbiont. Low levels were found of Al, Fe, Mn and Zn in nonmycorrhizal nematode-infected plants in low P soil. *G. intraradices*-inoculated plants reached the highest foliar levels of N, Ca, Mg, Mn, Cu and Zn. Mycorrhizal plants infected with *P. vulnus* maintained normal to high levels of Mn, Cu, and Zn. Inoculation with *G. intraradices* favours quince growth and confers protection against *P. vulnus* by improving plant nutrition.

Key words Arbuscular mycorrhizae
Cydonia oblonga · *Glomus intraradices* · Interaction
Pratylenchus vulnus · Quince

Introduction

Quince (*Cydonia oblonga* Mill.) rootstocks are widely used for pear cultivars in western Europe and, to a lesser extent, in the United States and eastern Europe. Quince offers important agronomic advantages, such as high productivity, reduced vigour, homogeneity, good adaptation to heavy soils, and resistance to many soil-borne diseases caused by bacteria, fungi and plant parasitic nematodes (Lombard and Westwood 1987). In Spain alone, approximately 80% of pear is grafted on quince rootstocks of the Angers and Provence types (Carrera 1990). The quince rootstock BA-29, a selection of Provence, has become popular among growers and has been found to be infected with the root-lesion nematode *Pratylenchus vulnus* Allen and Jensen in a recent survey conducted in fruit-tree nurseries in Spain (Pinochet unpublished work).

Pratylenchus vulnus is an important pest attacking many pome and stone fruit crops (McKelroy 1972; McKenry 1989; Scotto La Massese 1989) and has been found to be pathogenic on apple and pear (Fernández et al. 1992), almond (Marull et al. 1990), peach, peach-almond hybrid (Pinochet et al. 1991), and plum (Pinochet et al. 1993b).

Arbuscular mycorrhizal (AM) fungi colonize roots and improve plant nutrition, especially the uptake of phosphorus and other minor elements (Gerdemann 1968). A prophylactic effect of mycorrhizae has been often reported in the literature and it has been proved in many situations that mycorrhizae can reduce damage caused by pests and diseases (Dehne 1982; Perrin 1991). The symbiotic association is established in most fruit tree species and occurs naturally after seedlings are transplanted to undisturbed field sites.

Few studies have been concerned with nematode-fungi interactions with migratory endoparasitic nematodes and mycorrhizae on perennial crops. The beneficial effects on plant growth have been described by Smith and Kaplan (1988) on citrus seedlings infected with *Radopholus citrophilus* Huettel and the AM fungus *Glomus intraradices* Schenck and Smith, in relation to nonmycorrhizal plants infected with the nematode. More recently, Camprubí et al. (1993) found that *G. mosseae* increased tolerance of Marianna 2624 plum rootstock to damaging nematode levels in plants in-

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fected with *P. vulnus*. In another nematode-fungi interaction study, EMLA 26 apple rootstock responded favourably to mycorrhizal infection with *G. mosseae* in both the absence and presence of *P. vulnus* (Pinochet et al. 1993a). Mycorrhizae conferred protection against the nematode and increased capacity for nutrient uptake.

The importance of quince as a rootstock for pear and the lack of available information on the effects of both symbiont and pathogen on this host prompted the present investigation. This study reports the effects of *G. intraradices* and *P. vulnus* on plant development and nutritional response of micropropagated quince BA-29 rootstock inoculated with the AM fungus during the hardening phase that follows the weaning stage of in vitro plantlets.

Materials and methods

Micropropagated BA-29 quince rootstock was obtained from Agromillora Catalana SA, San Sadurní d'Anoia, Barcelona. In vitro plantlets were transferred from agar to 100-ml pots with fertilized sphagnum peat (TKS 1 Instant, Floragard GmbH), and acclimatized in a high-humidity chamber for 30 days. In February, plants were transferred to 6.7-l pots containing a pasteurized (75° C) sandy loam soil (80% sand, 18% silt, 2% clay), at pH 7.3, less than 2% organic matter content and a cation exchange capacity below 10 meq/100 g of soil. The phosphorus content of the soil at transplant fluctuated between 11 and 13 ppm, which is considered to be relatively low but not deficient and representative of soils dedicated to pear production in Spain.

An experiment with six treatments lasting one growing season was established: (1) control low in P (CLP); (2) control high in P (CHP); (3) plants in low-P soil inoculated with *P. vulnus* (Pv LP); (4) plants in low-P soil inoculated with *G. intraradices* (Gi LP); (5) plants in low-P soil with combined inoculations of *G. intraradices* and *P. vulnus* (Gi+Pv LP); (6) plants in high-P soil inoculated with *P. vulnus* (Pv HP).

During transplant to 6.7-l containers, plants from mycorrhizal treatments were inoculated with 10 g of *G. intraradices* soil inoculum. The inoculum consisted of rhizosphere soil from leek (*Allium porrum* L.) pot cultures containing heavily infected root fragments with many internal spores. The plants not receiving mycorrhizal inoculum received a filtrate of soil inoculum free from AM propagules.

Nematode treatments were inoculated with *P. vulnus* (simulating nursery field exposure of symbiont and pathogen) 2.5 months after mycorrhizal inoculation. A population of this nematode species was isolated from apple (*Malus silvestris* L.) in Girona, Spain, cultured monoxenically on carrot disks (Moody et al. 1973) and identified to the species level by the Commonwealth Institute of Parasitology, St. Albans, UK. Inoculum was recovered from cultures by adding sterile water and collecting the nematodes with a pipette. The volume of the nematode suspension was adjusted to deliver 1000 individuals per plant. Plants with uniform growth (±20 cm height and 10–12 leaves) were inoculated through six holes located 4–5 cm from the base of the stem. Containers were buried in the soil spaced 80 cm apart in a bucket microplot setup (Barker 1985) with 60% shade in field conditions until the conclusion of the study. Each treatment was replicated nine 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 P (0.10 g KH_2PO_4/l); plants from the P treatment received a double dose of P (0.20 g KH_2PO_4/l).

Plant growth (fresh top and root weights, stem diameter and shoot length) was measured at the end of the growing season (September 1993), 7 months after inoculation with *G. intraradices* and 4.5 months after nematode inoculation.

Nematodes in soil were recovered by differential sieving and sugar flotation (Jenkins 1964) from an homogenized 250-ml subsample (Pinochet et al. 1993b). 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 at 14500 rpm for 30 s given as 3 bursts of 10 s. 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.

The data on top weight, root weight, shoot diameter and shoot length were statistically analysed by ANOVA. Means were compared by Tukey's multiple range test ($P \leq 0.05$). The data on percentage of AM root colonization were transformed to arcsin for analysis.

Before harvest macro- and microelements were determined. Composite leaf samples from 5–6 midshoot leaves were taken early in August, avoiding senescent or necrotic tissue. Leaves were thoroughly washed in mild detergent, rinsed twice in distilled water and prepared for analysis. Samples were dehydrated in a temperature-controlled, fan-ventilated oven at 70±1° C for 48 h, ground in a ball mill and digested in wet acid (Jones et al. 1991) using nitric acid and perchloric acid. Analysis of all elements except nitrogen was performed with a Thermo Jarrell Ash inductively coupled plasma emission spectrometer (Munter and Grande 1981). Nitrogen content was determined with a Carlo Erba NA 1500 gas chromatograph.

Mycorrhizal infection was also assessed 7 months after inoculation. Root samples were collected and stained with 0.05% trypan blue in lactic acid, after the method of Phillips and Hayman (1970) as modified by Koske and Gemma (1989). The percentage AM root colonization was determined using the grid line intersect method (Giovanetti and Mosse 1980). From each mycorrhizal plant root, either inoculated or not with *P. vulnus*, 10-cm-long segments were excised after clarifying and staining the root. These fragments were mounted on calibrated slides and observed under a microscope. The number of internal vesicles formed by *G. intraradices* per mm of infected root was counted. The data obtained for all plants in each mycorrhizal treatment were analysed by ANOVA and Tukey's multiple range test. In order to observe more accurately any possible interaction 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 root tissues were digested, dehydrated to critical point in CO_2, mounted, sputtercoated with gold and examined at accelerating potentials of 8 V.

Results

The data on plant growth are summarized in Table 1. Mycorrhizae significantly increased top and root weights, shoot diameter and length of plants growing in low-P soil. Mycorrhizal plants from low-P soil had even higher top fresh weights and shoot lengths than nonmycorrhizal plants fertilized with a double dose of P. Inoculation of BA-29 plantlets with *G. intraradices* in low-P soil (Gi LP) was more effective than high P fertilization (CHP) in increasing top plant development. Differences between *P. vulnus* and control plants in low- and high-P soils were not significant.

Stem diameter and shoot length were higher in mycorrhizal plants infected by *P. vulnus* (Gi+Pv LP) than in nonmycorrhizal plants free from the pathogen (CLP). Plant development after 7 months was similar to that of noninoculated control plants in P-fertilized soil

Table 1 Growth response of BA-29 quince rootstock to inoculation with *Glomus intraradices* and *Pratylenchus vulnus* (1000 nematodes/plant) 7 and 4.5 months after arbuscular mycorrhizal (AM) fungus inoculation and nematode exposure, respectively. Data are means of nine replications. Means in the same column followed by the same letter do not differ according to Tukey's multiple range test ($P \leq 0.05$) (*LP* low phosphorus fertilization, *HP* high phosphorus fertilization)

Treatment	Fresh top wt. (g)	Stem diameter (mm)	Shoot length (cm)	Fresh root wt. (g)
Control LP	10.26 ab	4.63 a	56.55 ab	7.14 ab
Control HP	14.44 b	5.40 ab	70.00 bc	10.19 b
P. vulnus LP	8.81 a	4.87 a	50.22 a	6.07 a
P. vulnus HP	11.54 ab	5.04 ab	64.67 bc	7.52 ab
G. intraradices LP	19.50 c	5.91 b	89.39 d	10.05 b
G. intraradices + *P. vulnus* LP	14.50 b	5.83 b	77.30 cd	9.17 b

Table 2 Reproduction of *P. vulnus* and mycorrhizal root colonization by *G. intraradices* in BA-29 quince rootstock 7 months after inoculation with the AM fungus and 4.5 months after inoculation with 1000 nematodes/plant. Data are means of nine replications. Means in the same column followed by the same letter do not differ according to Tukey's multiple range test ($P \leq 0.05$) (*LP* low phosphorus fertilization, *HP* high phosphorus fertilization)

Treatment	Final nematode population (roots and soil)	Nematodes/g root	Percentage AM colonization	Number of vesicles/cm infected root fragment
P. vulnus LP	63420 a	7140 a	—	—
P. vulnus HP	82890 ab	8630 a	—	—
G. intraradices LP	—	—	91.8 b	12.8 b
G. intraradices + *P. vulnus* LP	139980 b	10049 a	65.4 a	4.7 a

Table 3 Mineral constituents of composite leaf samples from BA-29 quince rootstock inoculated with *P. vulnus* and *G. intraradices* 7 months after inoculation with the AM fungus and 4.5 months after inoculation with the nematode (*LP* low phosphorus fertilization, *HP* high phosphorus fertilization)

Treatment	Percentage dry wt.							ppm				
	N	Ca	Mg	Na	K	S	P	Al	Fe	Mn	Cu	Zn
Control LP	2.51	1.34	0.39	0.018	2.09	0.16	0.21	83	125	66	4.07	82.0
Control HP	2.48	1.44	0.39	0.016	2.28	0.17	0.19	85	196	66	3.35	66.6
P. vulnus LP	2.58	1.33	0.42	0.025	2.35	0.21	0.21	41	66	42	4.40	30.2
P. vulnus HP	2.47	1.24	0.36	0.018	2.25	0.16	0.17	64	143	65	3.97	96.5
G. intraradices LP	2.99	1.75	0.48	0.018	1.92	0.20	0.23	51	130	83	7.75	141.4
G. intraradices + *P. vulnus* LP	2.60	1.39	0.40	0.028	1.92	0.17	0.22	47	—	88	7.57	85.3

(CHP). All growth parameters measured were significantly higher in plants from the combined inoculation treatment (Gi+Pv LP) than in nonmycorrhizal plants infected with the nematode (Pv LP). In the presence of the nematode, P fertilization and inoculation with *G. intraradices* had similar effects on plant growth.

At the end of the experiment, the final nematode population recovered from plants with combined inoculations of pathogen and symbiont (Gi+Pv LP) was twofold larger than the population from plants inoculated with *P. vulnus* only in low-P soil (Pv LP). When plants received additional P fertilization (Pv HP), the difference was not significant.

AM colonization of quince was very high in plants inoculated only with *G. intraradices* (91.8±3.8%). The presence of *P. vulnus* caused a significant decrease in the extent of root infected by the fungus (65.4±6.4%). The mycorrhizal infection in nematode-inoculated plants was mainly arbuscular, with fewer internal vesicles formed within the roots (Table 2). SEM observations showed extensive colonization of both the nematode and the fungus in the same root fragments (Fig. 1). All nematode stages (eggs, larvae and adults) and typical cavity formation were present in the cortical parenchyma. Larval and adult forms were normally found aligned parallel to the axis of the root in young rootlets. Mycelium of *G. intraradices* and arbuscules colonizing cells were observed adjacent to nematode eggs.

Although no nutritional deficiencies were detected by foliar analysis, low levels of some elements were found in some treatments (Table 3). Nematode LP treatment showed lower concentrations of Al, Fe, Mn, and Zn, but always within sufficiency levels for quince. P fertilization of nematode-inoculated plants (Pv HP)

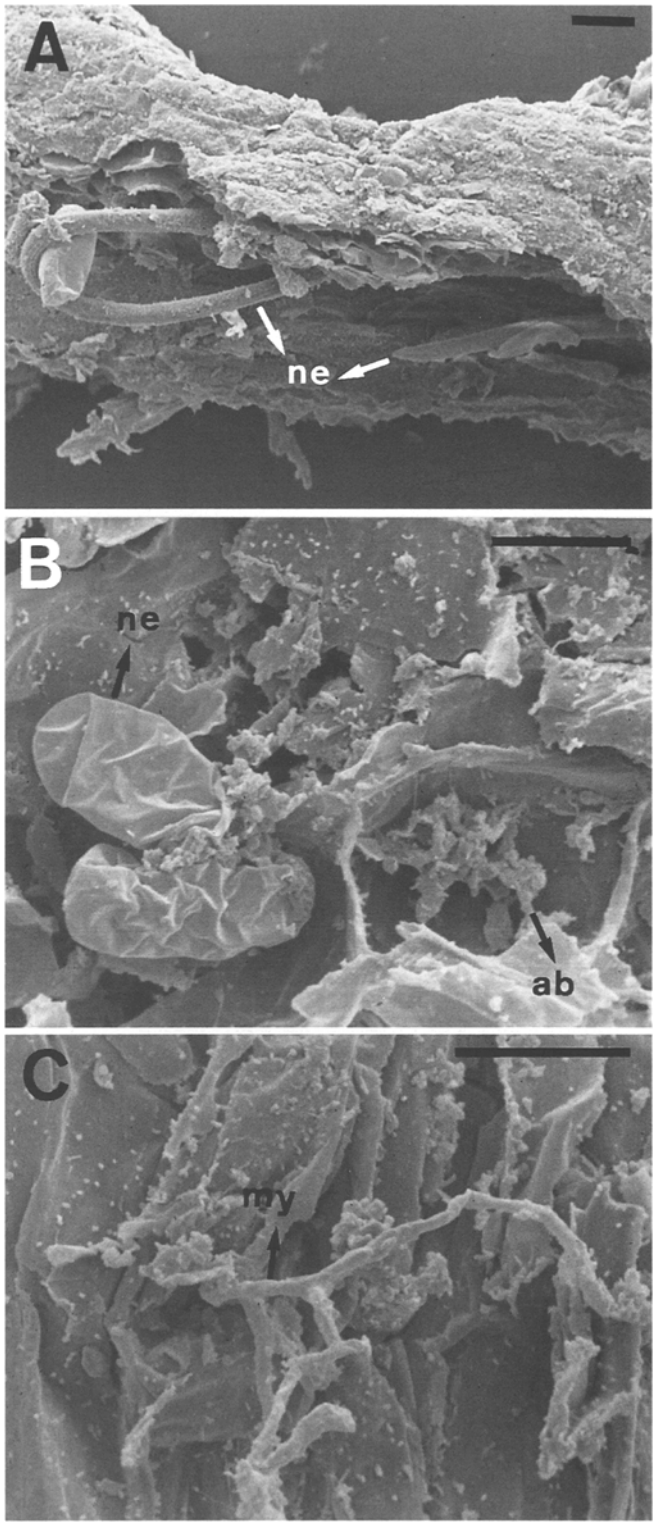

Fig. 1A–C Roots of BA-29 quince rootstock infected with the root-lesion nematode *Pratylenchus vulnus* and the arbuscular mycorrhizal fungus *Glomus intraradices*. **A** Root section showing specimens of *P. vulnus* emerging from cortical tissues; **B** nematode eggs (collapsed) adjacent to root cell with arbuscules of *G. intraradices*; *C* colonization of *G. intraradices* mycelium in cortical parenchyma (*ne* nematode, *ab* arbuscule, *my* mycelium). *Bar* 25 μm

resulted in higher foliar concentrations of these same elements. However, the highest K level was detected in Pv LP plants. *G. intraradices*-inoculated plants without the nematode reached the highest values for N, Ca, Mg, Mn, Cu, and Zn. Levels for S were low in general.

Discussion

Seven months after inoculation, the host roots were almost completely colonized by the AM fungus, and mycorrhizal plants had higher top weights and shoot lengths than plants fertilized with additional P. *C. oblonga* BA-29 rootstock is highly dependent on mycorrhizal infection with *G. intraradices*. P fertilization is less effective than mycorrhizae at increasing plant growth.

A large population build-up of *P. vulnus* was recorded at harvest. Plant growth (fresh top and root weights and shoot length) was affected and root destruction caused by *P. vulnus* but differences between Pv LP and CLP plants were not significant 4.5 months after inoculation with the nematode, suggesting that a longer exposure to the pathogen was needed to damage the plant. Although quince is a suitable host, it appears to be less susceptible to *P. vulnus* than apple and pear rootstocks (Fernández et al. 1992).

Mycorrhizal plants infected by the root-lesion nematode grew better than control plants free from pathogen in low-P soil, and were not significantly different from control plants fertilized with a high dose of P. These results prove that the AM fungus *G. intraradices* alleviated damage caused by *P. vulnus* to plant development. A prophylactic effect of mycorrhizae against soil-borne pathogens has been reported for many host plants and situations (Perrin 1991).

In a recent review, Francl (1993) reported mixed results on interactions between mycorrhizae and nematodes. There are very few references to migratory endoparasitic nematodes which mention the beneficial effect of the symbiosis (O'Bannon and Nemec 1979; Umesh et al. 1988). The results obtained with BA-29 show that an additional P fertilization had the same effect on plant development as inoculation with *G. intraradices* after 4.5 months growth in the presence of *P. vulnus* and support the hypothesis.

The higher number of nematodes in mycorrhizal plants is related to an increased root mass production, since the number of nematodes/g root did not vary. Fresh root weight was significantly higher in mycorrhizal plants infected with *P. vulnus* than in nonmycorrhizal plants, a difference that can account for the twofold increase in final nematode population at harvest. This also indicates that the beneficial effects of mycorrhizal fungi are indirect and apparently linked to improved nutrient status of the infected host plant due to its larger root system.

There are contradictory results in the literature, where some dual-inoculation studies report a reduction

of endoparasitic nematodes in mycorrhizal plants (Francl 1993), and Ingham (1988) suggests that endoparasitic nematodes and AM fungi are mutually inhibitory. The assessment of internal infection achieved by *G. intraradices* showed that there was a detrimental effect of the root-lesion nematode on the extent of root colonized by the mycorrhizal fungus on quince rootstock. This effect had been observed before on Myrobalan and Marianna plum rootstocks, where *G. mosseae* Gerd. and Trappe achieved 52% and 72% infection of *P. vulnus*-free plants compared with 18% and 52% in nematode-inoculated plants, respectively (Camprubí et al. 1993). The percentage of root length colonized by *G. intraradices* in quince infected by *P. vulnus* was still high, above 60%, but the invasion of the root tissue by the nematode negatively affected the establishment of the fungus within the host root. Both organisms seem to compete for space within the root, as they colonize the root cortex and depend on the host plant for adequate nutrition.

SEM observations and quantification of internal vesicles under the light microscope proved that the morphological features of the internal colonization changed when the parasite invaded host roots. In plant roots infected by both symbiont and nematode, the fungal infection was mainly of the arbuscular type; very few vesicles were seen within the root segments. In contrast, vesicles were abundant in the roots of mycorrhizal plants not inoculated with *P. vulnus*. In these plants, vesicles were predominant in the root cortex after 7 months growth. Similar observations on the effects of root endoparasitic nematodes on AM development have been reported before. Suresh and Bagyaraj (1984) noticed a reduction in root colonization of nematode-infected tomato (*Lycopersicon esculentum* Mill.) plants; Umesh et al. (1988) reported the same result in banana (*Musa acuminata* Colla.) and in *Citrus limon* L. mycorrhizal roots, vesicles were not formed when plants were infected by *Radophulus similis* (Cobb) Thorne (O'Bannon and Nemec 1979). *G. mosseae* developed normally in feeder roots of *C. limon,* but in the presence of *Tylenchulus semipenetrans* Cobb vesicles were not formed (O'Bannon et al. 1979).

Nutrient exchange between plant and fungus takes place in the interphase of the arbuscule and the host cell wall, the arbuscular infection being formed by metabollically active fungal structures and living root cells (Dexheimer et al. 1979). Vesicles are storage structures with very large amounts of lipids produced in the older regions of infection. The nematode causes drainage of nutrients from the host plant due to feeding and migration. In mycorrhizal plants, the fungus is in the active phase of exchange, with many arbuscules, probably to compensate the detrimental effect of the parasite. The protective role of mycorrhizae is indirect through improved plant nutrition, with the morphology of the internal infection adapted to the host nutrient demands.

Mosse (1973) described morphological changes in the AM infection at high levels of P. Sanders and Tinker (1973) quantified arbuscules and noticed that their number was lower in high-P conditions. The carbohydrate supply to the endophytes might also be important in determining fungal establishment (Jasper et al. 1979) and factors which might affect the carbohydrate status of the roots can affect mycorrhizal colonization. Carbohydrates formed by photosynthesis provide energy for respiration and synthesis of metabolites and it has been estimated that the mycorrhizal symbiont utilizes 15% of host photosynthates (Harley and Smith 1983). A low supply of carbohydrates to the fungus probably brings about an increase in the number of arbuscules and a decrease in total AM colonization percentage according to Daft and El-Giahmi (1978) and Gunze and Hennessy (1980).

It has been shown that photosynthesis is usually reduced by nematodes (Wallace 1987). *P. vulnus* destroys parenchymatic tissue from the host root and feeds on the living host cells. The level of carbohydrates in the root decreases as a result of the nematode infection and the mycorrhizal fungus would remain in the metabolically active arbuscular phase, an adaptation to maintain plant health in response to infection by root-lesion nematode. The protective effect of mycorrhizae is exerted through improvement of host nutrition, and the morphology of the fungal infection seems to adapt to the situation in the presence of the parasite.

Nematode LP treatment produced a markedly lower foliar concentration of Fe, Mn, and Zn. However, Mn and Zn were twice as high in mycorrhizal plants inoculated with *P. vulnus*, indicating that absorption and transport of both elements was not impaired and that the plant is capable of maintaining normal to high levels of both elements in spite of high nematode densities in the roots. Cu showed a similar trend for *G. intraradices*-inoculated treatments with and without *P. vulnus*, underlining the beneficial effects of enhanced nutrition due to early mycorrhizal infection.

We conclude that early inoculation of micropropagated plants with *G. intraradices* increases host tolerance to the root-lesion nematode *P. vulnus* on BA-29 quince rootstock. Mycorrhizae compensate for the root disruption caused by the pathogen, allowing the plant to continue functioning in spite of damaging levels of the nematode. The results add to the information on the significance of mycorrhizae for the development of rootstocks infected with migratory endoparasitic nematodes, so often associated with replant problems in fruit tree crops (Nyzcepir and Halbrendt 1993), and will contribute to a better understanding of the interaction.

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