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# Growth and nutritional response of Nemared peach rootstock infected with *Pratylenchus vulnus* and the mycorrhizal fungus *Glomus mosseae*

Jorge PINOCHET, Cinta CALVET, Amelia CAMPRUBI and Carolina FERNANDEZ

*Departamento de Patología Vegetal, Institut de Recerca i Tecnologia Agroalimentàries, IRTA, Crta. de Cabrils s/n, 08348 Cabrils, Barcelona Spain.*

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**Summary** – The effects of the interaction between *Pratylenchus vulnus* and the endomycorrhizal fungus *Glomus mosseae* on growth of Nemared peach rootstock were studied under microplot conditions for two growing seasons. Fresh shoot weights, stem diameter, shoot length, and fresh root weights of nematode inoculated plants colonized or not by *G. mosseae* were significantly lower than those of nematode free plants. High levels of phosphorus in non-mycorrhizal controls enhanced plant growth. Mycorrhizal treatments with *P. vulnus* showed significantly lower final nematode population and number of nematodes per gram of root compared with the non-mycorrhizal treatment with *P. vulnus*. Mycorrhizal colonization was not affected by the presence of the nematode. Cu was the only deficient element detected by foliar analysis, although low levels were found for Fe in nematode infected plants. The highest levels of Na, Mg, Mn, and Zn were detected in *P. vulnus* inoculated plants. Mycorrhizal plants had the highest values for Cu and Al. *G. mosseae* favours Nemared peach growth but does not confer protection against *P. vulnus*.

**Résumé** – ***Croissance et réponse aux nutriments des porte-greffe de pêcher " Nemared " infestés par* Pratylenchus vulnus *et le champignon mycorrhizien* Glomus mosseae** – Les effets de l'interaction entre *Pratylenchus vulnus* et le champignon mycorrhizien *Glomus mosseae* sur les porte-greffe de pêcher " Nemared " ont été étudiés en microparcelles pendant deux saisons de croissance. Le poids frais des pieds, le diamètre de la tige, la longueur des pieds et le poids frais des racines sont significativement plus faibles chez les pieds infestés par le nématode – qu'ils soient ou non colonisés par *G. mosseae* – que chez les pieds non infestés. Un taux élevé de phosphore augmente la croissance des pêchers dans le cas de témoins non mycorrhizés. Les traitements des pieds infestés par *P. vulnus* à l'aide de mycorrhizes provoquent une diminution de la population finale du nématode et du nombre de nématodes par gramme de racine par rapport aux pieds infestés par *P. vulnus* et non traités à l'aide du champignon. La colonisation par les mycorrhizes n'est pas affectée par la présence du nématode. Chez les pieds infestés par le nématode, le Cu est le seul élément déficitaire détecté par analyse foliaire, quoique des taux faibles de fer y aient été observés. Les taux les plus élevés de Na, Mg, Mn et Zn ont été détectés chez les pieds infestés par *P. vulnus*. Les pieds mycorrhizés recèlent les taux les plus élevés de Cu et d'Al. *G. mosseae* est bénéfique pour la croissance des pêchers " Nemared " mais ne leur confère aucune protection contre *P. vulnus*.

**Key words :** Arbuscular mycorrhizae, *Glomus mosseae,* interaction, lesion nematode, peach, *Pratylenchus vulnus, Prunus persica,* rootstock.

The root-lesion nematode *Pratylenchus vulnus* is a major pest attacking pome and stone fruit crops in warm Mediterranean environments (Mc Elroy, 1972; Scotto la Massèse, 1989; McKenry, 1989). This nematode is present in Spain in nurseries and commercial orchards and is pathogenic on apple and pear (Fernández *et al.,* 1992), almond, peach, peach-almond hybrid and plum (Pinochet *et al.,* 1991; 1993 *b*).

Arbuscular mycorrhizal fungi (AM) are obligate symbionts that increase nutrient uptake by plants, especially phosphorus and other minor elements (Gerdemann, 1968; Smith, 1987). Arbuscular mycorrhizae benefit plants under physiological stress, such as drought conditions (Nelson, 1987), nutrient deficient soils (Linderman, 1988), and when attacked by diseases (Dehne, 1982; Perrin, 1991) and nematodes (Smith, 1987; Francl, 1993). This symbiotic association is established in virtually all fruit tree species and occurs naturally in nursery stock (early infection) or when seedlings are transplanted to the field. Some fruit tree species are more mycorrhizal dependent than others (Powell, 1984), even though AM fungi have a low specificity for the host (Daniels-Hetrick, 1984).

An increased interest in the use of the root-knot nematode resistant peach " Nemared " and the reported benefits of AM fungi on peach growth and nutrition (Gilmore, 1971; Lambert *et al.,* 1979) encouraged studies to determine if early mycorrhizal inoculation of peach seedlings in the nursery would confer some degree of protection when established in *P. vulnus* infested soils at a stage when plants are most vulnerable. Recent studies in Spain have shown the importance of early

mycorrhizal infection on plant growth in "Marianna 2624 " and " Myrobalan 605 " plum rootstocks (Camprubí *et al.*, 1993), and on the apple rootstock EMLA 26 (Pinochet *et al.*, 1993 *a*) infected with *P. vulnus*. Early mycorrhizal inoculation of peach established in *P. vulnus* infested soil could increase nutrient availability to the plant in the presence of the nematode. The purpose of this investigation was to study the effects of *P. vulnus* on plant growth and nutrition in AM fungus *Glomus mosseae* (Nicol & Gerd.) Gerd. & Trappe inoculated and non-inoculated seedlings of Nemared peach rootstock.

## Materials and methods

Seeds of Nemared peach [*Prunus Persica* (L.) Batsch] rootstock were stratified at 5 °C for 70 days in perlite filled trays and were moved to a greenhouse to induce seed germination. Germinated seeds were transferred to 6.7 $dm^3$ containers tilled with a pasteurized sandy soil (88 % sand, 10 % silt, 2 % clay), pH 7.62, and less than 2 % organic matter.

A two-year experiment with five treatments was established :

1) control low in phosphorus (CLP)
2) control high in phosphorus (CHP)
3) plants inoculated with *P. vulnus* low in phosphorus
4) plants inoculated with *G. mosseae* (AM fungus) low in phosphorus
5) plants with joint inoculations of *G. mosseae* and *P. vulnus* low in phosphorus.

During transplant to 6.7 $dm^3$ containers, AM treatments were inoculated with *G. mosseae*. Each plant received 5 g of soil inoculum from onion (*Allium cepa* L.) and clover (*Trifolium repens* L.) pot cultures, containing spores and infected root fragments with 0.63 propagules/g measured by the most probable number technique (MPN) (Daniels & Skinner, 1982). Non-mycorrhizal plants received a filtrate of soil inoculum free from AM propagules. Phosphorus content in the soil at transplant fluctuated between 11 and 13 ppm, considered low, but representative of soils of many fruit tree growing areas of Spain. Four months after AM inoculation, and prior to nematode inoculation (simulating nursery-to-field exposure of symbiont inoculated seedlings and nematode pathogen), root samples were collected and stained with 0.05 % trypan blue in lactic acid (Phillips & Hayman, 1970) modified by the procedure described by Koske and Gemma (1989) to determine if AM root colonization had occurred (positive-negative).

*Pratylenchus vulnus* was isolated from rose (*Rosa multiflora* L.) in Cabrils, Barcelona, Spain, and cultured monoxenically on carrot discs (Moody *et al.*, 1973). Identification to species level was made 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 nematode suspension was adjusted to give 500 individuals per plant (equivalent to 74 nematodes per kg of soil), delivered through five holes located at 4-6-cm distance from the base of the stem. Containers were buried in the soil spaced at 80 cm apart in a bucket microplot setup (Barker, 1985) in an open shadehouse in field conditions until the end of the study. 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 & Arnon, 1950) nutrient solution low in phosphorus (0.10 g $PO_4KH_2$/l) once a week, except for the control with high phosphorus (CHP) in which higher levels of phosphorus (0.18 g $PO_4KH_2$/l) were added.

Plant growth (fresh top weight, shoot diameter and shoot length) were recorded at the end of the first season, and 5 months after nematode inoculation (October 1992). The same parameters plus fresh root weights were assessed in the second growing season 16 months after nematode inoculation (August-September 1993).

Nematodes in soil were recovered by differential sieving and sugar flotation (Jenkins, 1964) from a homogenized 250 $cm^3$ subsample (Pinochet *et al.*, 1993 *b*). Nematodes in roots were extracted by cutting the whole root system into pieces (*ca* 1 cm long), and macerating it with water in a blender at 14 500 rpm for 30 s given as 10 s intervals. A small root sample (approximately 20 % of the whole root system) was used to estimate percentage of AM root infection. 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 infection at the end of the second season, root samples were collected and stained as previously described. The percentage of AM colonisation was determined using the grid line intersect method (Giovanetti & Mosse, 1980). Size sample was sufficient to count at least 150 intersects per sample.

Data on fresh shoot weight, root weight, stem diameter, shoot length and nematode reproduction were analyzed by a one-way ANOVA. Means were compared by Fisher's LSD test ($P \leq 0.05$).

Before harvest, P and micro-elements in plant tissue were determined. Composite leaf samples from ten to twelve midshoot leaves were taken in mid summer. Leaves were washed in mild detergent, rinsed thoroughly in distilled water and prepared for analysis. Samples were then dehydrated in a temperature controlled fan ventilated oven at 70 ± 1 °C during 48 h. Following dehydration, dry leaves were ground in a ball mill and digested in wet acid (Jones *et al.*, 1991) using nitric and perchloric acid. Analysis for all elements was made with a Thermo Jarrell Ash inductively coupled plasme (ICP) emission spectrometry (Munter & Grande, 1981).

## Results

At the end of the first season (1992), fresh shoot weights of both *P. vulnus* inoculated treatments were significantly lower than *G. mosseae* and CHP treatments but not different from CLP treatment (Table 1). For

***Table 1.*** *Fresh shoot weights, shoot length, stem diameter, and root weights of Nemared peach rootstock inoculated with 500* Pratylenchus vulnus *per plant alone and in combination with* Glomus mosseae *5 (first season) and 16 months (second season) after nematode inoculation.*

| Treatment* | First season (1992) | | | Second season (1993) | | | |
|---|---|---|---|---|---|---|---|
| | Fresh shoot weight (g) | Stem diameter (mm) | Shoot length (cm) | Fresh shoot weight (g) | Stem diameter (mm) | Shoot length (cm) | Fresh root weight (g) |
| Control low P | 23.6 *ab* | 6.9 *abc* | 164.2 *abc* | 36.7 *b* | 7.4 *a* | 139.3 *a* | 29.9 *a* |
| Control high P | 36.8 *a* | 7.9 *a* | 246.5 *a* | 50.5 *a* | 8.2 *a* | 160.8 *a* | 39.2 *a* |
| *P. vulnus* low P | 10.1 *b* | 5.7 *c* | 82.5 *c* | 19.7 *c* | 5.7 *b* | 104.8 *b* | 16.2 *b* |
| *G. mosseae* low P | 30.0 *a* | 7.2 *ab* | 211.3 *ab* | 39.6 *ab* | 7.8 *a* | 156.5 *a* | 35.8 *a* |
| *G. mosseae* + *P. vulnus* low P | 14.9 *b* | 5.9 *bc* | 120.5 *bc* | 22.0 *c* | 6.0 *b* | 116.5 *b* | 18.6 *b* |

* Data are means of eight replications. Means in the same columns followed by the same letter do not differ according to Fisher's LSD test ($P > 0.05$).

stem diameter and shoot length, CHP reached the highest values and differed from nematode inoculated treatments. Plants inoculated with *G. mosseae* alone also differed from nematode inoculated plants but not from mycorrhizal plants in combination with the nematode. No differences were found between CLP, CHP and mycorrhizal treatments. In the second season (1993), fresh shoot weights of *P. vulnus* inoculated treatments were lower than *G. mosseae,* CLP and CHP treatments. CHP differed from CLP but not from *G. mosseae* inoculated plants. *P. vulnus* treatments with and without mycorrhiza also showed less plant growth in comparison to the CLP, CHP and *G. mosseae* treatments for stem diameter, shoot length, and fresh root weight at harvest.

***Table 2.*** *Reproduction of* Pratylenchus vulnus *and percentage of mycorrhizal root colonization by* Glomus mosseae *alone and in combination in Nemared peach rootstock 20 months after inoculation with the AM fungus and 16 months after inoculation with 500 nematodes per plant.*

| Treatment* | Final population per plant (soil) and roots) | Nematodes per g of root | *Pf/Pi*** | Mycorrhizal colonization (%)*** |
|---|---|---|---|---|
| *P. vulnus* | 41 740 *a* | *1780 a* | 84 | - |
| *P. vulnus* + *G. mosseae* | 24 330 *b* | *1030 b* | 49 | 26 + 8 |
| *G. mosseae* | - | - | - | 27 + 6 |

* Data are means of eight replications. Means in the same columns followed by the same letter do not differ according to Fisher's LSD test ($P < 0.05$).
** *Pf/Pi* : Final population/initial population (nematode reproduction rate).
*** Data are means of eight replications + standard deviation.

The mycorrizal treatment with *P. vulnus* showed a significantly lower final nematode population and amount of nematodes per gram of roots in relation to plants inoculated with *P. vulnus* alone, resulting in suppressed nematode reproduction ($P < 0.05$). Nematode multiplication rate *(Pf/Pi)* in the mycorrhizal treatment was 49 vs 84 in non-mycorrhizal plants inoculated with *P. vulnus* (Table 2). At harvest, the percentage of root colonization by *G. mosseae* was similar in both *G. mosseae alone* (27 %) and *G. mosseae* with *P. vulnus* treatments (26 %). Both organisms colonized the same root tissues (Fig. 1).

The only deficient element in foliar analysis was Cu in both nematode inoculated treatments (Table 3). Low

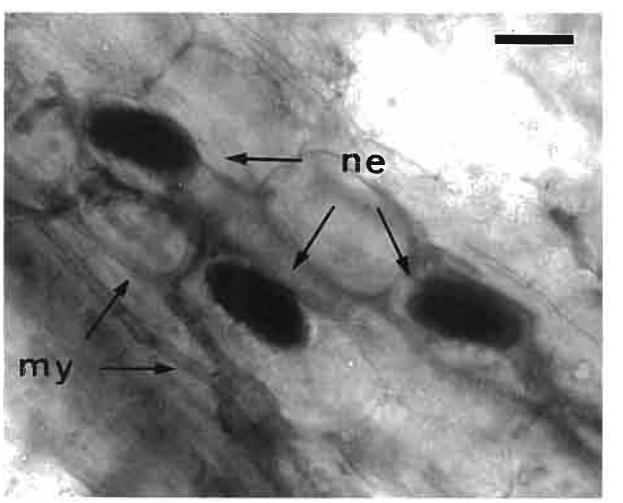


***Fig. 1.*** *Eggs of* Pratylenchus vulnus *(ne) and mycelium of* Glomus mosseae *(my) colonizing the same root tissues in Nemared peach rootstock* (Bar scale = 40μm).

***Table 3.*** *Mineral constituents of composite leaf samples from Nemared peach rootstock inoculated with* Pratylenchus vulnus *and* Glomus mosseae *alone and in combination 16 months after inoculation with the nematode.*

| Treatment | Percentage of dry weight | | | | | ppm | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Ca | Mg | Na | S | P | Al | Fe | Mn | Cu | Zn |
| Control low P | 0.64 | 0.39 | 0.009 | 0.11 | 0.07 | 95 | 258 | 286 | 2.43 | 61.5 |
| Control high P | 0.73 | 0.41 | 0.010 | 0.13 | 0.15 | 82 | 181 | 226 | 2.42 | 48.5 |
| *P. vulnus* low P | 0.76 | 0.45 | 0.017 | 0.13 | 0.08 | 96 | – | 470 | 1.64 | 76.8 |
| *G. mosseae* low P | 0.64 | 0.39 | 0.008 | 0.12 | 0.08 | 114 | 195 | 308 | 2.94 | 35.5 |
| *G. mosseae* + *P. vulnus* low P | 0.69 | 0.43 | 0.013 | 0.13 | 0.09 | 96 | 166 | 363 | 1.42 | 75.8 |

levels of some elements, mainly Ca, Na, and P for peach, were found in some treatments. Most elements were within sufficiency levels (Jones *et al.,* 1991). Leaves of plants inoculated with the nematode were low in Cu and Fe. The addition of P in CHP treatment resulted in increased plant growth and gave the highest P values. However, P fertilization did not result in higher foliar concentrations of any of the eleven remaining elements, whereas in *G. mosseae* inoculated plants resulted in increased foliar levels of Al and Cu. On the other hand, Mg, Mn, Na, and Zn reached the highest values in *P. vulnus* inoculated plants.

## Discussion

There were significant differences ($P < 0.05$) between *P. vulnus* mycorrhizal plants and nematode inoculated plants without *G. mosseae* in final nematode populations and in the number of nematodes per gram of root (Table 2). This suggests that root colonization by *G. mosseae* had a negative effect on the development of *P. vulnus*. The mechanisms of nematode suppression are unknown but would seem to be related to physiological changes induced by the symbionts affecting the nematode's food source rather than direct competition for space. Another AM fungus, *Gigaspora margarita* Becker & Hall, has been shown to reduce the host suitability of *Pratylenchus brachyurus* on cotton (Hussey & Roncadori, 1978). In spite of a lower level of nematode infection in mycorrhizal plants, the prophylactic effects that mycorrhizae could have over the nematode colonization in Nemared peach would seem minor or none, since nematode inoculated treatments, with and without mycorrhiza, showed similar stunting, especially during the second growing season (Table 1). This would agree with previous results obtained with the same nematode and AM fungus on San Julian 655-2 plum rootstock (Camprubí *et al.,* 1993) in which mycorrhizal infection had little overall effect on plant growth. In both cases the mycorrhizal colonization was low after two seasons of exposure to the AM fungus. In contrast, *G. mosseae* enhanced growth on the plum rootstock Marianna 2624 in the presence of *P. vulnus* during the first year as compared to the nematode inoculated treatment alone in that same study. In a more recent AM-nematode interaction study with the same pathogen and symbiont, the apple rootstock EMLA 26 responded favourably to mycorrhizal infection in the presence of *P. vulnus;* mycorrhiza conferred significant protection against this pathogen and an increased capacity for nutrient uptake (Pinochet *et al.,* 1993 *a*). EMLA 26 mycorrhizal colonization was higher (37 %) than in the present study. Similar beneficial effects on plant growth have been described by Smith and Kaplan (1988) on citrus seedlings infected with another migratory endoparasitic nematode, *Radopholus citrophilus,* and the AM fungus *Glomus intraradices* Schenck & Smith, in relation to non-mycorrhizal plants infected with the nematode. The authors concluded that increased plant growth and fewer citrus burrowing nematode in mycorrhizal and high phosphorus levels in the plants was a direct result of increased phosphorus nutrition of the host. These opposed situations suggest that the ability of the nematode to colonize root tissue in areas where the AM fungus has established would depend mainly on the mycorrhizal dependency of the host, as well as on the AM fungus isolate used (Granger *et al.,* 1983).

Mycorrhizal plants had a similar percentage of root colonization in nematode inoculated *versus* nematode free plants (26 *vs* 27 %, respectively), indicating that *P. vulnus* had no direct negative effect over *G. mosseae* colonization after 16 months of exposure to the nematode. Also, the low percentage of mycorrhizal colonisation by *G. mosseae* indicates reduced dependency of Nemared peach rootstock to this particular endophyte.

Our findings indicate that some nutrient elements decrease (Fe and Cu) while others increase notably in leaf tissues (Mg, Mn, Zn, and Na) in nematode inoculated treatments. In the first case, absorption and transport of

Fe and Cu to aerial parts would seem to be impaired by the destruction of the root cortical tissues caused by the nematode probably due to the loss of the capacity for differential permeability which reduces nutrient element transport (Kirkpatrick, 1964). In contrast, Mg, Mn, Zn and Na, seem to be absorbed continuously and accumulate in leaf tissues as a result of reduced growth, thus their increasing concentration. The lower concentrations in leaf tissues of these same elements in treatments without the nematode is explained by a growth dilution effect (Kleinschmidt & Gerdemann, 1972; Granger *et al.*, 1983). A similar pattern for these elements (increase in Zn, Mg, and Mn and reduction in Fe and Cu in foliar levels) has been described on rose (Sher, 1957) and apple (Pinochet *et al.*, 1993 *a*) in plants infected with *P. vulnus*.

This study concludes that Nemared peach responds favourably to mycorrhizal infection by *G. mosseae* in the absence of *P. vulnus*. Inoculation with the AM fungus does not confer protection against nematode damage, even though nematode population was partially suppressed by *G. mosseae*. Neither does the AM fungus confer an increased capacity for nutrient uptake as observed with other *Prunus* and *Malus* rootstocks infected with this migratory endoparasitic nematode in their early stages of plant growth (Camprubí *et al.*, 1993; Pinochet *et al.*, 1993). Stroebel *et al.* (1982), reported that increased fertilization and mycorrhizae (*Glomus etunicatum* Becker & Gerdemann and *Gigaspora margarita*) can increase tolerance to peach sedlings infected with the root-knot nematode *Meloidogyne incognita*. Discrepancies between this study and ours are probably due to different experimental conditions : peach cultivars, AM fungus evaluated, and specially, the different feeding habits of *M. incognita* and *P. vulnus*.

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