

Pathogenicity of *Pratylenchus vulnus* on plum rootstocks

Jorge PINOCHET *, Joan MARULL *, Rodrigo RODRIGUEZ-KABANA **, Antonio FELIPE ***, and Carolina FERNANDEZ *

* Departamento de Patología Vegetal, IRTA, Crta. de Cabrils s/n, 08348, Cabrils, Barcelona, Spain,

** Department of Plant Pathology, Auburn University, Auburn, AL 36849, USA, and

*** Departamento de Fruticultura, Servicio de Investigación Agraria, Apartado 727, 50080 Zaragoza, Spain.

Accepted for publication 13 January 1993.

Summary – The effects of *Pratylenchus vulnus* on development of five commercial plum rootstocks were evaluated in greenhouse and microplot experiments. Three rootstocks, PSM 101, Montizo, and Citation are new introductions into the Spanish market. In a greenhouse test, Montizo, PSM 101, Citation and Saint Julien 655-2 were good hosts for *P. vulnus*. Root weights of uninoculated Montizo and Saint Julien were higher ($P \leq 0.05$) than for inoculated plants, whereas fresh top weights of uninoculated Citation and Montizo were significantly higher than those of inoculated plants. Only Montizo showed a significant increase in shoot length in uninoculated over inoculated plants. In a microplot experiment lasting 28 months, inoculation with *P. vulnus* reduced top and root weights of PSM 101, Marianna 2624 and Saint Julien 655-2 but did not affect trunk diameter. Parasitism was high for all rootstocks fluctuating from 2890 (Marianna) to 7220 (PSM 101) nematodes per gram of root. Plants without nematodes began growth following winter dormancy earlier than those that were inoculated. Saint Julien was the rootstock most susceptible to *P. vulnus*.

Résumé – Pathogénie de *Pratylenchus vulnus* envers des porte-greffe de prunier – Les effets de *Pratylenchus vulnus* ont été évalués, en serre et en microparcelles, sur cinq porte-greffe de prunier dont PSM 101, Montizo et Citation, récemment introduits sur le marché espagnol. En serre, Montizo, PSM 101, Citation et Saint Julien 655-2 se sont montrés hôtes de *P. vulnus*. Le poids des racines de Montizo et de Saint Julien est plus élevé ($P \leq 0.05$) chez les pieds inoculés avec le nématode que chez les pieds témoins, non inoculés; il en est de même pour le poids frais des parties aériennes de Citation et de Montizo. La longueur de la tige n'est supérieure chez les plants témoins que pour Montizo. Lors d'une expérience en microparcelles (28 mois), les poids des parties aérienne et racinaire des porte-greffe inoculés PSM 101, Marianna 2624 et Saint Julien 655-2 sont inférieurs à ceux des témoins, mais il n'y a aucune différence en ce qui concerne le diamètre de la tige au collet. Le niveau de parasitisme est élevé : il varie de 2890 à 7220 nématodes par gramme de racines pour Marianna et PSM 101, respectivement. Les plants non inoculés croissent plus rapidement après la latence hivernale. Saint Julien paraît plus sensible à *P. vulnus* que les autres porte-greffe testés.

Key-words : Pathogenicity, *Pratylenchus vulnus*, plum, *Prunus* spp., rootstocks.

The root lesion nematode *Pratylenchus vulnus* Allen & Jensen attacks several pome and stone fruit crops in Spain (Pinochet *et al.*, 1991; Fernández *et al.*, 1992). Although its distribution is unknown in the country, this nematode species seems to be very destructive where present. It is also widespread in France and Italy where it is considered a severe pathogen of many pome, nut and stone fruit crops (Scotto La Massèse, 1975, 1989; In-serra *et al.*, 1979). Most of the information on damage caused by this nematode in plum (*Prunus* spp.) has been obtained in California (Day & Serr, 1953; McElroy, 1972; McKenry, 1987, 1988; Culver *et al.*, 1989) where this pest is ubiquitous and important. Most plum rootstocks used in California are different from those available in Spain. However, in recent years, several new introductions into the Spanish market from the United States, France, and Spanish research institutes have become quite popular with growers. Of special interest are the plum "Pollizos" Montizo and PSM 101 and the American rootstock Citation (Felipe, 1989; Socías, 1990). The first two have desirable agronomic features

and adapt well to some of the harsh mediterranean conditions (dry land, calcareous soils, and low fertility).

The purpose of this research was to determine the pathogenicity of *P. vulnus* on five plum rootstocks recently introduced into the Spanish market.

Materials and methods

The rootstocks Citation, Saint Julien 655-2, Marianna 2624, Montizo, and PSM 101 (Table 1) were obtained from the Departamento de Fruticultura del Servicio de Investigación Agraria de la Diputación General de Aragón, Zaragoza, and two private sources. All the materials with exception of PSM 101 were propagated from hardwood cuttings. These were treated with a 40 % alcohol solution containing 1500 ppm of indolebutyric acid for 20 s to induce root growth. Cuttings were transplanted into small 200 cm³ pots containing a 5:1 (v:v) sand – peat mixture, and placed in a greenhouse for rooting. PSM 101 was propagated *in vitro* by a commercial source and was delivered bare root during winter. A greenhouse and a microplot experiment were es-

established at the same time. The rootstocks Saint Julien 655-2 and Marianna 2624 were used as reference rootstocks (Pinochet *et al.*, 1991).

Table 1. Description of five plum rootstocks tested against *Pratylenchus vulnus* in Spain.

Rootstock	Species or selection	Origin *
Citation	<i>Prunus persica</i> × <i>P. belsiana</i>	Zaiger, California, U.S.A.
Montizo	<i>P. insititia</i>	SIA, Zaragoza, Spain
Saint Julien 655-2	<i>P. insititia</i>	INRA, France
PSM 101	<i>P. insititia</i>	CSIC, Zaragoza, Spain
Marianna 2624	<i>P. cerasifera</i> × <i>P. munsoniana</i>	University of California Davis, California, U.S.A.

* SIA : Servicio de Investigación Agraria; INRA : Institut National de la Recherche Agronomique; CSIC : Consejo Superior de Investigaciones Científicas.

GREENHOUSE EXPERIMENT

The effects of *P. vulnus* on the rootstocks Citation, Saint Julien 655-2, Montizo and PSM 101 were evaluated for fresh top weight, root weight, shoot length and nematode reproduction at 15 months after inoculation. Both rooted cuttings and *in vitro* rootstocks were transplanted to 2.6 L PVC pots that contained a pasteurized sandy loam soil (73 % sand, 22 % silt and 5 % clay), pH 7.5, less than 1 % organic matter content and a cation exchange capacity (C.E.C.) of less than 10 meq/100 g of soil. Plants were kept in the greenhouse for 3 to 4 months before nematode inoculation (June).

A *P. vulnus* population isolated from rose (*Rosa multiflora* L.) in Cabriels, Barcelona was cultured monoxenically on carrot (*Daucus carota* L.) discs (Moody *et al.*, 1973). The identification to species level was made by the Commonwealth Institute of Parasitology, St. Albans, United Kingdom. Inoculum of *P. vulnus* was recovered from stock cultures by adding water to the cultures and collecting the nematodes on a 0.025-mm screen (500 mesh-inch). The volume of the nematode suspensions was adjusted to give 1000 individuals per plant. Plants with uniform growth were inoculated through four holes in the potting mixture located at 4 to 5 cm distance from the base of the plant. Inoculated pots were placed in a sand bed to minimize temperature and humidity fluctuations. Temperature conditions in the greenhouse fluctuated between 6 and 14 °C in winter which resulted in the induction of dormancy, and 13 to 32 °C the rest of the year.

MICROPLOT EXPERIMENT

Hardwood cuttings from Marianna 2624, PSM 101, and Saint Julien 655-2 were placed in an unheated

greenhouse for rooting. Rooted material was transplanted individually into 32 cm diameter-bucket microplots (Barker, 1985) containing a sandy textured soil (75 % sand, 19 % silt and 6 % clay), pH 7.3, less than 2 % organic matter, and a C.E.C. of less than 12 meq/100 g of soil. Microplots were established in a shaded area (45 % shade) in the field for 28 months and were set at 1 m spacing. Three months after transplant (late spring), nematodes were inoculated in the same manner as for the greenhouse experiment. Plants were watered as needed and fertilized with full-strength Hoagland's (Hoagland & Arnon, 1950) nutrient solution once a week throughout the growing season.

Data on fresh top weights, length of shoots and trunk diameter were measured at the end of each growing season. Trunk diameter measurements were made at 5 cm from the ground. Fresh root weights, final nematode population and numbers of nematodes per gram of root were assessed at the end of each experiment. Soil from each pot 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, stirred thoroughly. Nematodes were extracted from a 250 cm³ subsample of the slurry by differential sieving using 150, 74 and 38 µm screens (100, 200 and 400 mesh-inch, respectively) and sugar flotation (Jenkins, 1964). Fresh root weights were determined and the whole root system was cut in small pieces (1 cm long) and macerated with water in a blender for 30 s given at 10 s intervals. Nematode suspension was then concentrated using 150, 74, and 25 µm sieves (100, 200, and 500 mesh-inch, respectively). Root tissue and debris collected on the 150 µm sieve were discarded. Nematodes were recovered in the remaining sample by sugar flotation.

Data on plant emergence following winter dormancy in the early spring season of the second year were taken every 20 days starting in January 10 until April 1. A bud sprouting to 1 cm length was considered to have broken dormancy.

Each rootstock was represented by seven replications (pots) in the first experiment and eight microplots in the second experiment arranged in a completely randomized design. Data on nematode reproduction and plant growth were analyzed by a one-way ANOVA. When F values were significant, differences between means were evaluated for significance using Duncan's multiple range test ($P \leq 0.05$).

Scanning electron microscope observations were conducted to complement the greenhouse and microplot studies. Selected root pieces from active growing tissue of parasitized Citation and PSM 101 rootstocks 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 aluminium stubs and sputtercoated with

gold. The material was examined at accelerating potentials of 8, 10, and 15 kV.

Results

GREENHOUSE EXPERIMENT

All the tested rootstocks were good hosts for *P. vulnus*, although higher population densities were found in Saint Julien 655-2 (209 880) than in Montizo (43 200) or PSM 101 (78 060) (Table 2). PSM 101 had lower numbers of nematodes per gram of fresh root weight than Montizo and Saint Julien 655-2. Root weights were higher in uninoculated Montizo and Saint Julien 655-2 than in corresponding inoculated material. Top weights of uninoculated Citation and Montizo were higher than those for *P. vulnus* inoculated treatments (Table 3). Montizo was the only rootstocks that showed a significant increase in shoot length in uninoculated over inoculated plants.

Table 2. Reproduction of *Pratylenchus vulnus* on four plum rootstocks 15 months after inoculation with 1000 nematodes per plant in a greenhouse experiment.

Rootstock *	Final population per plant (soil and roots)	Nematodes per g of root
Montizo	43 200 a	1860 b
PSM 101	78 060 a	640 a
Citation	92 580 ab	1190 ab
Saint Julien 655-2	203 880 b	4350 b

* Data are means of seven replications. Actual data are presented, but data were transformed to $\log_{10}(x+1)$ for analysis. Means in the same columns followed by the same letter do not differ according to Duncan's multiple range test ($P \leq 0.05$).

Table 3. Fresh root, top weights and shoot length of four plum rootstocks 15 months after inoculation with 1000 *Pratylenchus vulnus* per plant in a greenhouse experiment.

Growth parameters	Treatment	Rootstock *			
		Citation	PSM 101	Montizo	Saint Julien 655-2
Root weights (grams)	Control	6.56 a	25.04 a	11.41 a	11.74 a
	<i>P. vulnus</i>	4.12 a	18.18 a	4.95 b	7.26 b
Top weights (grams)	Control	2.45 a	6.49 a	3.71 a	4.65 a
	<i>P. vulnus</i>	0.91 b	5.49 a	1.83 b	3.42 a
Shoot length (cm)	Control	18.64 a	40.63 a	19.90 a	36.66 a
	<i>P. vulnus</i>	12.20 a	30.19 a	9.20 b	32.60 a

* Data are means of seven replications. Means in the same columns followed by the same letter do not differ according to Duncan's multiple range test ($P \leq 0.05$).

Table 4. Reproduction of *Pratylenchus vulnus* on three plum rootstocks 28 months after inoculation with 1000 nematodes per plant in a microplot experiment.

Rootstock *	Final population per plant (soil and roots)	Nematodes per g of root
Marianna 2624	177 210 a	2890
Saint Julien 655-2	210 110 a	4640
PSM 101	708 320 b	7220
		NS

* Data are means of eight replications. Actual data are presented, but data were transformed to $\log_{10}(x+1)$ for analysis. Means in the same columns followed by the same letter do not differ according to Duncan's multiple range test ($P \leq 0.05$).

Table 5. Fresh top weights, shoot length, trunk diameter, and fresh root weights of three plum rootstocks evaluated under microplot conditions at 16 and 28 months after inoculation with 1000 *Pratylenchus vulnus* per plant.

Growth parameter	Treatment	First year (1991)			Second year (1992)		
		Marianna 2624	Saint Julien 655-2	PSM 101	Marianna 2624	Saint Julien 655-2	PSM 101
Top weights (grams)	Control	17.80 a	15.46 a	27.43 a	76.87 a	55.36 a	70.79 a
	<i>P. vulnus</i>	13.50 a	3.79 b	19.47 b	52.10 b	31.86 b	49.69 b
Shoot length (cm)	Control	124.90 a	97.05 a	132.56 a	330.90 a	173.70 a	298.60 a
	<i>P. vulnus</i>	123.60 a	30.70 b	106.36 b	285.90 a	123.20 b	236.70 a
Trunk diameter (mm)	Control	8.7 a	6.6 a	8.8 a	11.6 a	9.2 a	11.0 a
	<i>P. vulnus</i>	8.6 a	4.7 b	8.0 a	10.5 a	7.3 a	9.6 a
Root weight (grams)	Control	-	-	-	70.44 a	47.76 a	76.89 a
	<i>P. vulnus</i>	-	-	-	34.76 b	22.90 b	47.69 b

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

MICROPLOT EXPERIMENT

The three plum rootstocks tested in this trial were good hosts for the nematode, although final population

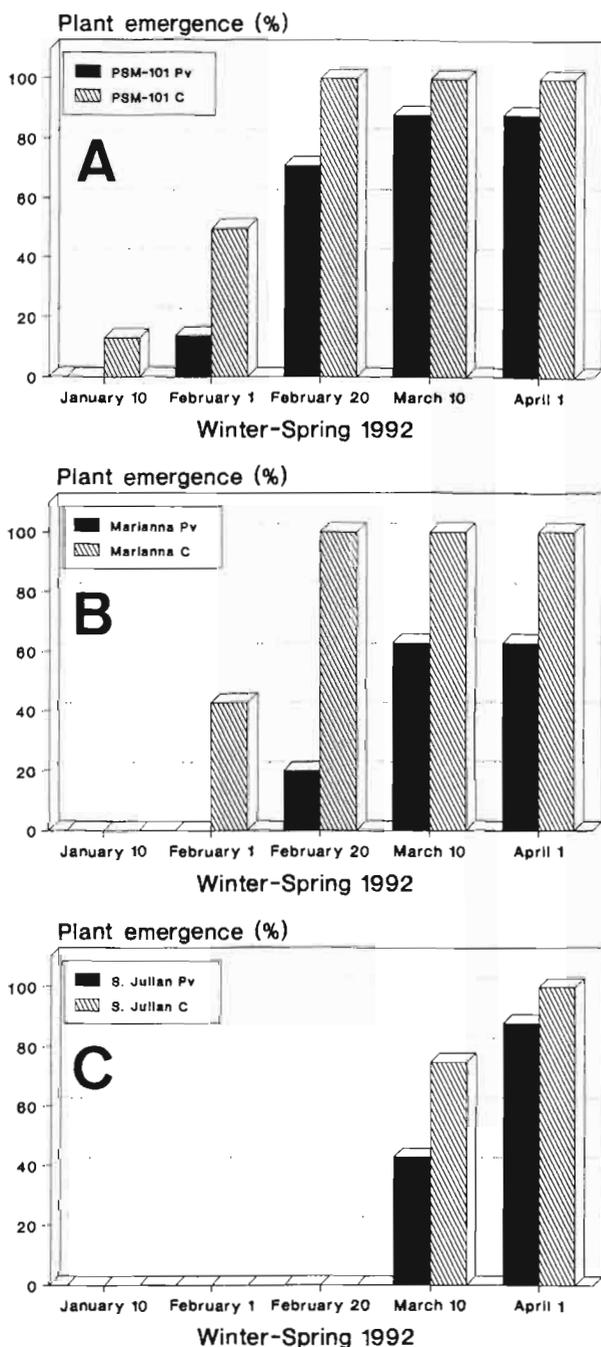


Fig. 1. Plant emergence after winter dormancy in *Pratylenchus vulnus* inoculated (Pv) and non-inoculated (C) plum rootstocks. A : PSM 101; B : Marianna 2624; C : Saint Julien 655-2.

densities were higher in PSM-101 (707 320) than for Marianna 2624 (177 210) or Saint Julien 655-2 (210 110). There were no differences in the numbers of nematodes per gram of root among these rootstocks (Table 4). During the first year, there were no differences for top weights, shoot length and trunk diameter between inoculated and uninoculated treatments in Marianna 2624 as was the case for Saint Julien 655-2. Inoculated PSM 101 showed significantly reduced top weights and shoot length but no difference in trunk diameter (Table 5). In the following growing season (second year), both inoculated Marianna 2624 and PSM 101 differed in top weights and root weights from uninoculated treatments, but not in shoot length and trunk diameter. Inoculated Saint Julien 655-2 evidenced significantly reduced values for top weights, shoot length and root weights and no difference in trunk diameter.

EMERGENCE FROM DORMANCY

Uninoculated plum rootstocks showed a higher percentage of plant emergence following winter dormancy in the second year than to *P. vulnus* inoculated plants (Fig. 1). The greatest difference between the number of dormant and emerged plants was observed with Marianna 2624. PSM 101 was the first to emerge and Saint Julien 655-2 the last (around 60-day difference between both rootstocks).

SEM observations revealed extensive colonization of *P. vulnus* with formation of cavities in the cortical parenchyma of the roots of Citation and PSM 101 (Fig. 2). The nematode was usually found aligned parallel to the root axis, although in some cases it showed no defined orientation within the root tissue. The nematode was generally found in young active growing roots. *P. vulnus* was not detected in the stele.

Discussion

Results of this study indicate that *P. vulnus* is a true pathogen of the five tested plum rootstocks, capable of causing significant root destruction and reduction in plant growth as a result of a high and rapid population build-up in the first years. In the greenhouse experiment Montizo was severely affected, whereas in the microplot experiment, plant growth was relatively more reduced in Saint Julien 655-2 during both years indicating it was the most susceptible of the three rootstocks.

The rootstocks Montizo and PSM 101 known as "Pollizo" plums are two recent Spanish selections well adapted to the poor agronomic conditions that prevail in the mediterranean region (Cambra, 1979; Felipe *et al.*, 1989). Both evidence medium vigour and early production. They are resistant to root asphyxia, iron chlorosis induced by calcareous soils and are compatible with the

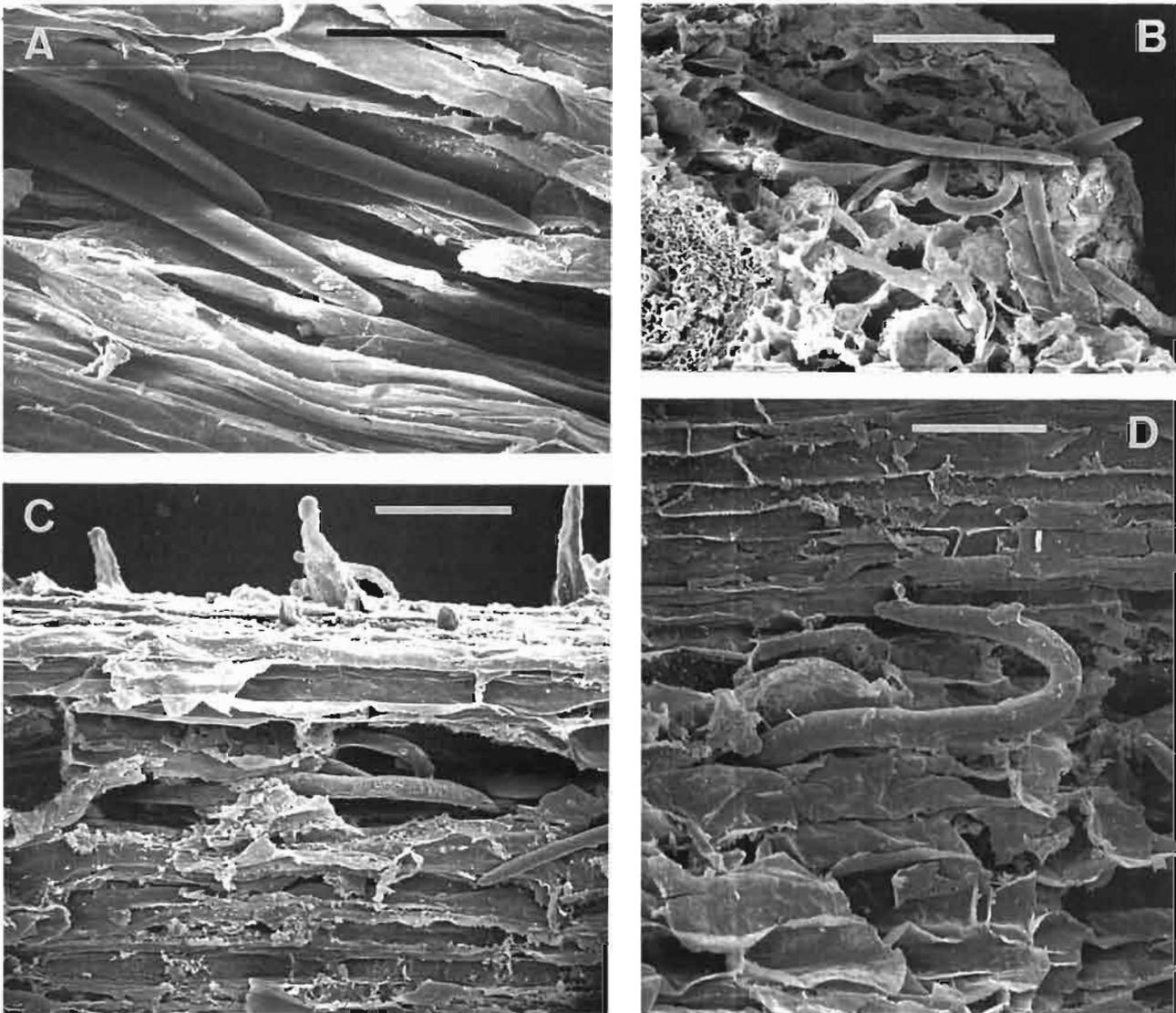


Fig. 2. Roots of plum rootstocks infected with *Pratylenchus vulnus*. A : Larval and adult stages aligned parallel to the root axis in root of Citation plum; B : Cross-section of Citation root showing nematodes colonizing the cortical tissues; C : Longitudinal section of PSM 101 root with nematode in cavities; D : Specimen emerging from ruptured cells of the cortical parenchyma of PSM 101 root. Cells of the endodermis (upper portion) appear without damage. (Bar scale : A, C, D = 50 μm ; B = 100 μm).

majority of almond, apricot, peach and plum varieties. In previous evaluations, both rootstocks have also shown to be resistant to two root-knot nematode species, *Meloidogyne incognita* (Kofoid & White) Chitwood (Pinochet *et al.*, 1990) and *M. javanica* (Treb) Chitwood (Pinochet *et al.*, 1992). In this study, Montizo showed the highest reduction in plant growth and should be considered the most susceptible of the tested rootstocks (Table 3). In contrast, PSM 101 was the only rootstock not significantly affected by *P. vulnus* suggesting that it could be a tolerant rootstock. However, when evaluated under mi-

croplot conditions, PSM 101 evidenced reductions in top and root weights (21 and 38 %, respectively) indicating that a longer period of time was needed to express damage. In general, its growth was less affected by *P. vulnus* than that of Marianna 2624, especially during the second year, although PSM 101 should be regarded as a more suitable host for *P. vulnus* (Table 4). Its estimated level of tolerance remains to be determined and should be measured in terms of yield. Marianna 2624 has been considered tolerant to *P. vulnus* in terms of production in California (McKenry, 1988).

In a field study conducted in California, Culver *et al.* (1989), found significant reductions in relative trunk diameter increase in four *Prunus* genotypes that included Myrobalan 29 C plum, at 175 days after planting in the field with an inoculum of six *P. vulnus* per gram of soil. In the study, top and root weights did not generally reflect nematode damage. Our results indicate that only inoculated Saint Julien 655-2 showed a significant decrease ($P \leq 0.05$) in trunk diameter during the first growing season, but not in the second, even though there was a slight decrease (not significant) in trunk diameters in all three rootstocks. In contrast, top and root weights, and in some cases shoot length reduction, were important indicators of damage.

The differences recorded in plant emergence following winter dormancy is perhaps the most unique finding of this study (Fig. 1). Sprouting was retarded in all *P. vulnus* inoculated rootstocks for about one month in comparison to uninoculated rootstocks. This delay could affect yield negatively by delaying physiological activity of the plant. This shortened growth period is likely to be compounded later in the season by further destruction of the root system by the nematode.

In general, damage observed at the histological level in plum root caused by *P. vulnus* is comparable to that found in other fruit tree crops (Corbett, 1974; Marull & Pinochet, 1991; Fernández *et al.*, 1992).

Acknowledgements

The authors thank Dr. Juan Negueroles for providing plant material. This work was financed by the Spanish Instituto Nacional de Investigaciones Agrarias, INIA, Grant No 8528.

References

- BARKER, K. R. (1985). Design of greenhouse and microplot experiments for evaluation of plant resistance to nematodes. In: Zuckerman, B. M., Mai, W. F. & Harrison, M. B. (Eds). *Plant nematology laboratory manual*. Amherst, U.S.A. Univ. Massachusetts agric. Exp. Statn.: 107-113.
- CORBETT, D. C. M. (1974). *Pratylenchus vulnus*. C. I. H. Descriptions of Plant-parasitic Nematodes Set 3, No 37. Commonw. Inst. Helminth., 4 p.
- CAMBRA, R. (1979). Selección clonal de "Pollizo de Murcia". *Informac. técn. econ. agr.*, 36: 21-30.
- CULVER, D. J., RAMMING, D. W., & MCKENRY, M. V. (1989). Procedures for field and greenhouse screening of *Prunus* genotypes for resistance and tolerance to root-lesion nematode. *J. Am. Soc. hort. Sci.*, 114: 30-35.
- DAY, L. H. & SERR E. F. (1953). Comparative resistance of rootstocks of fruit and nut trees to attack by a root-lesion or meadow nematode. *Proc. Am. Soc. hort. Sci.*, 57: 150-154.
- FELIPE, A. J. (1989). *Patrones para frutales de pepita y hueso*. Barcelona, España, Ediciones Técnicas Europeas, 181 p.
- FELIPE, A. J., BLASCO, A. B., CARRERA, M., & GELLA, R. (1989). "Monpol" 645 y "Montizo" 646: Nuevas selecciones clonales de "Pollizo" de Murcia. *Informac. técn. econ. agrar.*, 83: 41-46.
- FERNANDEZ, C., PINOCHET, J., & DOLCET, R. (1992). Host parasite relationship of *Pratylenchus vulnus* on apple and pear rootstocks. *Nematropica*, 22: 227-236.
- HOAGLAND, D., & ARNON, D. I. (1950). The water culture method for growing plants without soil. *Calif. agric. exp. Statn., Circ. No 347*, 32 p.
- INSERRA, R. N., ZEPP, A., & VOLVAS, N. (1979). I *Pratylenchus* dell'Italia meridional. *Nematol. medit.*, 7: 137-162.
- JENKINS, W. R. (1964). A rapid centrifugal flotation technique for separating nematodes from soil. *Pl. Dis. Repr.*, 48: 692.
- MARULL J., & PINOCHET, J. (1991). Host suitability of *Prunus* rootstocks to four *Meloidogyne* species and *Pratylenchus vulnus* in Spain. *Nematropica*, 21: 185-195.
- MCÉLROY, F. D. (1972). Nematodes of tree fruits and small fruits. In: Webster, J. M. (Ed.). *Economic nematology*. London, Academic Press: 335-376.
- MCKENRY, M. V. (1987). Survey of nematodes associated with almond production in California. *Pl. Dis.*, 71: 71-73.
- MCKENRY (1988). Nematodes. In: La Rue, J. H. & Scott Johnson, R. (Eds). *Peaches, plums and nectarines. Growing and handling for fresh market*. Coop. Ext., Univ. Calif., Div. Agric. nat. Resources, Publ., No 3331: 139-147.
- MOODY, E. H., LOWNSBERY, B. F., & AHMED, J. M. (1973). Culture of the root lesion nematode *Pratylenchus vulnus* on carrot disks. *J. Nematol.*, 19: 125-134.
- PINOCHET, J., MARULL, J., VERDEJO, S., SOLER, A., & FELIPE, A. (1990). Evaluación de patrones de ciruelo, pollizo, membrillero y peral al nematodo de las agallas, *Meloidogyne incognita* (Kofoid y White) Chitwood. *Boh. Sanid. veg. Plagas*, 16: 717-722.
- PINOCHET J., VERDEJO, S., & MARULL, J. (1991). Host suitability of eight *Prunus* spp. and one *Pyrus communis* rootstocks to *Pratylenchus vulnus*, *P. neglectus* and *P. thornei*. *J. Nematol.*, 23: 570-575.
- PINOCHET, J., MARULL, J., & FELIPE, A. (1992). Respuesta de patrones de melocotonero, ciruelo, y cerezo de reciente introducción en España a *Meloidogyne javanica*. *Nematropica*, 22: 99-102.
- SCOTTO LA MASSÈSE, C. (1975). Tests d'hôtes de quelques porte-greffe et variétés fruitières à l'égard de *Pratylenchus vulnus* Allen et Jensen. *C.r. Séanc. hebd. Acad. Agric. France*, 61: 1088-1095.
- SCOTTO LA MASSÈSE, C. (1989). Les problèmes posés par les nématodes phytophages à l'amandier. In: Felipe, A. J. & Socias, R. (Eds). *Options méditerranéennes*. Séminaire du GREMPA sur les porte-greffe de l'amandier, CIHEAM, Zaragoza, España: 33-38.
- SOCIAS, R. (1990). *Estado actual de los patrones frutales*. Asoc. Interprof. Desarrollo Agrario (AIDA), Volumen Extra 9, Zaragoza, España, 255 p.