HOST SUITABILITY OF *PRUNUS* ROOTSTOCKS TO FOUR *MELOIDOGYNE* SPECIES AND *PRATYLENCHUS VULNUS* IN SPAIN[†]

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ABSTRACT

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Ten almond varieties and three experimental peach-almond hybrids were evaluated for their reactions against *M. arenaria, M. incognita, M. javanica, M. hapla,* and *Pratylenchus vulnus.* In a first experiment, four almond varieties commonly used as rootstocks in Spain were susceptible to *M. incognita, M. arenaria,* and *M. hapla.* In a second experiment, the almond D-3-5, and the hybrids G × N No 9 and Cachirulo showed varying degrees of resistance to *M. javanica.* The remaining cultivars were susceptible to *M. javanica.* In a third experiment, all tested materials were good hosts of *P. vulnus.* The peach-almond hybrid G × N No 1, highly resistant to several species of root-knot nematodes, had the highest level of parasitism by *P. vulnus.* In an histological study, *P. vulnus* colonized extensively the cortical parenchyma of the roots of Garrigues, Garfí and G × N No 1 forming large pockets that contained all stages of the nematode as well as cavities.

Key words: Almond, host-parasite relationship, *Meloidogyne arenaria, M. incognita, M. javanica, M. hapla,* peach, peach-almond hybrid, *Pratylenchus vulnus, Prunus amygdalus, P. persica,* resistance.

RESUMEN

Marull, J. y J. Pinochet. 1991. Capacidad hospedadora de patrones de *Prunus* a cuatro especies de *Meloidogyne* y *Pratylenchus vulnus* en España. Nematrópica 21:185–195.

Diez variedades de almendro y tres híbridos experimentales de melocotonero × almendro fueron evaluados frente e *Meloidogyne incognita, M. arenaria, M. javanica, M. hapla,* y *Pratylenchus vulnus.* En un primer ensayo, cuatro variedades de almendro, comunmente utilizadas como patrones francos en España, fueron susceptibles a *M. incognita, M. arenaria,* y *M. hapla.* En un segundo ensayo, el almendro D-3-5 y los híbridos G × N No 9 y Cachirulo mostraron diferentes niveles de resistencia a *M. javanica.* El resto de los cultivares de almendro evaluados en este ensayo fueron susceptibles a *M. javanica.* En un tercer ensayo, todos los materiales resultaron ser buenos hospedadores de *P. vulnus.* La selección G × N No 1, altamente resistente a varias especies de *Meloidogyne,* presentó el mayor incremento poblacional de *P. vulnus.* En un estudio histpatológico en Garrigues, Garfí y G × N No 1, se observó que *P. vulnus* colonizó de manera extensiva el parenquima cortical de las raíces formando bolsas que contenían todos los estadíos del nematodo e indujo a la formación de cavidades.

Palabras clave: almendro, híbrido de melocotonero × almendro, melocotonero *Meloidogyne arenaria, M. incognita, M. javanica, M. hapla, Pratylenchus vulnus, Prunus amygdalus, P. persica,* relación parásito hospedador, resistencia.

INTRODUCTION

Almond (*Prunus amygdalus* Batsch) and peach (*Prunus persica* Stock.) are important fruit tree crops planted on 670 000 ha of cultivated land in Spain (7). Almond is primarily grown in dryland conditions characterized by poor and calcareous soils while peach is generally cul-

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tivated in irrigated land where better agronomic practices are implemented. Seedlings from natural crosses or selfs have been used traditionally to propagate almond and peach, especially almond. However, in the last decade, there has been a rapidly expanding utilization of peach-almond hybrids, which possess agronomic advantages over non-hybrid seedlings, such as more vigor and phenotypic homogeneity.

The presence of *Meloidogyne* spp. in nurseries and orchards appears to be common in Spain (1,6,10). Losses caused by root-knot nematodes in peach and almond production are unknown, although they likely are similar to those that occur in other production areas that share similar environmental and agronomic conditions (3,12,15,19). The main nematode control practices for establishing an orchard are preplant fumigation or a 1-year fallow following a cereal crop. Several root-knot resistant peach rootstocks, like Nemaguard, Hansen, and Nemared, are also commercially available, but some of these materials do not always adapt well to Mediterranean growing conditions. Some introduced rootstocks tend to suffer badly from iron chlorosis and root asphyxia, and from root diseases caused by *Phytophthora, Verticillium, Armillaria,* and *Agrobacterium tumefasciens* (8,22).

In the last three years an effort was made to identify almond and peach-almond hybrid rootstocks with resistance to root-knot nematodes (9,17). Several interesting peach-almond hybrids in their early stages of selection that originated from breeding programs of Spanish research centers have shown a high degree of resistance to *Meloidogyne incognita* (Kofoid and White) Chitwood and *M. arenaria* (Neal) Chitwood (14). The responses of some of these materials and other new germoplasm introductions, to other root-knot species and to the lesion nematode *Pratylenchus vulnus* Allen and Jensen, are unknown. The latter pest is also found in fruit tree orchards but apparently with less frequency than root-knot species.

The purpose of this study was to evaluate the reaction of 10 almond varieties, three of which are commonly used as rootstocks in Spain, and three experimental peach-almond hybrids to *M. incognita, M. arenaria, M. hapla, M. javanica* (Treub) Chitwood, and *P. vulnus* under greenhouse conditions.

MATERIALS AND METHODS

Seeds of the almond varieties Atocha, Garrigues, Desmayo Largueta, and Marcona were supplied by the Departamento de Arboricultura Mediterránea of IRTA, Mas Bové in Reus, Tarragona. Seeds of the almond varieties Desmayo Rojo, Guara, Garfí, Tardive de la Verdiere, Tuono, and D-3-5, seeds of the hybrid Cachirulo, and cuttings of the hybrids G × N No 1, and G × N No 9 were provided by the Programa de Fruticultura del Servicio de Investigación Agraria de la Diputación General de Aragón in Zaragoza. The principal agronomic features of each material are given in Table 1.

Seeds were treated with a 5% solution of copper oxychloride for 24 hours, rinsed with running water to eliminate traces of the fungicide, covered with moist paper towel, and stratified in perlite trays. These trays were kept in a storage room at 4 C for 45 days, and then seed material was moved to an ambient temperature greenhouse to induce germination. The peach-almond hybrids were propagated from wood cuttings. Cuttings were treated for 6-10 seconds

Table 1. General information on 10 almond varieties and three peach-almond hybrids evaluated against four *Meloidogyne* species and *Pratylenchus vulnus* in Spain.

Cultivar	Species	Selection/origin	Main agronomic features
Atocha	*P. amygdalus*	Unknown, originally from Murcia, Spain	Good vigor. Used as rootstock. Medium productivity.
Desmayo Largueta	*P. amygdalus*	Unknown, originally from Tarragona, Spain	Medium vigor. Widely used variety. Medium productivity. Sensitive to frost.
Desmayo Rojo	*p. amygdalus*	Unknown, originally from Aragón, Spain	Good vigor. Used as rootstock. Medium productivity. Adapted to dryland conditions.
Garrigues	*P. amygdalus*	Unknown, originally from Murcia, Spain	Good vigor. Widely used as rootstock. High productivity. Well adapted to dryland conditions. Sensitive to frost.
Marcona	*P. amygdalus*	Unknown, originally from Alicante, Spain	Medium vigor. Widely used variety. High productivity. Requires good soil and cultural practices.
Tuono	*P. amygdalus*	Unknown, originally from Apulia, Italy	Good vigor. Self-compatible variety. Late variety. Resistant to frost.
Guara	*P. amygdalus*	Apparently descendant from Tuono	Good vigor. Self-compatible variety. High productivity. Late variety. Resistant to frost.
Garfí	*P. amygdalus*	Selection of Garrigues S.I.A. Zaragoza, Spain	Good vigor. Easy propagation. High productivity. Adapted to dryland conditions.
Tardive de la Verdiere	*P. amygdalus*	Unknown, originally from Provence, France	Low vigor. Unfrequent variety. High productivity.
D-3-5	*P. amygdalus*	Titan × Tuono S.I.A., Zaragoza, Spain	Good vigor. Self-compatible. Late variety. Small seed.
Cachirulo	*P. persica* × *P. amygdalus*	R. Red Leaf × D. D'Aurons S.I.A., Zaragoza, Spain	Poor vigor. Colored leaf.
G × N No 1	*P. persica* × *P. amygdalus*	Nemared × Garfí S.I.A. Zaragoza, Spain	Good vigor. Colored leaf. Easy propagation. Resistant to *M. incognita* and immune to *M. arenaria*.
G × N No 9	*P. persica* × *P. amygdalus*	Nemared × Garfí S.I.A., Zaragoza, Spain	Good vigor. Colored leaf. Easy propagation. Immune to *M. arenaria*.

with a 50% hydroalcoholic solution that contained 2 000 ppm of indole butyric acid. Cuttings then were planted in small pots (200 cm^3) containing a 3:1 (v:v) sand and peat mixture previously pasteurized at 80 C. The germinated seeds and the rooted cuttings were transplanted to 2-L containers filled with a pasteurized sandy loam textured soil (73% sand, 18% loam) with pH 7.6, < 1% organic matter, and a cation exchange capacity < 10 meq/100 g soil. Plants were kept in the greenhouse for 6 weeks before inoculation. The five nematode species were originally collected from different localities in Catalonia, Spain: *M. incognita* from almond in Reus, Tarragona; *M. hapla* from kiwi (*Actinidia deliciosa*) in Cabrils, Barcelona; and *M. javanica* from fig (*Ficus carica*) in Cabrils, Barcelona; *M. arenaria* from tomato (*Lycopersicon esculentum*) in Canet, Barcelona; and *M. javanica* from fig (*Ficus carica*) in Cabrils, Barcelona. These four root-knot isolates were increased on tomato cv. Roma from single egg mass cultures. The *P. vulnus* population was isolated from rose (*Rosa multiflora*) in Cabrils and reared monoxenically on carrot disk cultures (16).

Three experiments were carried out, one in 1989 and two in 1990. The reaction of the almond varieties, Atocha, Desmayo Largueta, Marcona, and Garrigues were evaluated to *M. arenaria, M. incognita,* and *M. hapla* in the first experiment. Plants with uniform growth of approximately 30 cm height (25 to 30 leaves) were inoculated with a suspension of 2 000 eggs per plant through 4 holes in the soil 3 cm from the base of the plant. Inoculum was prepared by macerating infested tomato roots in a blender for 10 sec in a 0.12-0.15% solution of NaOC1 to dissolve egg masses. Eggs were concentrated in a 0.025-mm-pore sieve (500 mesh) and rinsed with tap water before inoculation (2).

In the second experiment, the almond varieties Garrigues, Desmayo Rojo, T. Verdiere, Garfí, Tuono, Guara, and D-3-5, and the hybrids Cachirulo and G × N No 9 were evaluated against a population of *M. javanica.* These same materials, with the exceptions of Desmayo Rojo and G × N No 9, were tested in a third experiment inoculated with *P. vulnus.* In both experiments, the inoculation level was 1 000 nematodes per container. *Meloidogyne javanica* inoculum was prepared in the same way as in the first experiment. For inoculum preparation in the experiment with *P. vulnus,* nematode populations in carrot disk culture jars were recovered with distilled water and diluted for aliquot preparation. In this study, Garrigues almond was used as the susceptible control for the four root-knot nematode species. The second and third experiments were initiated 30 days apart.

Plants were harvested 120 days after inoculation. Gall indices, final nematode population per plant in soil and in roots, and the numbers of nematodes per gram of root were determined for root-knot nematodes. Gall index was determined by using the 1-6 scale recommended by Barker (2) for evaluating resistance to *Meloidogyne* spp.: 1 = 0; 2 = 1–10; 3 = 11–30; 4 = 31–70; 5 = 71–90; and 6 = more than 91 galls per root system. Nematodes in soil were washed free of soil particles in a second pan with a known volume of water. Contents of both pans were mixed and stirred for 1 min and 250 cm^3 of the resulting slurry was used as a sample. Nematodes then were extracted by differential sieving and sugar flotation (11). Nematode densities in the soil were calculated based on 250-cm^3 aliquots. Nematodes in the roots were extracted in the same manner as inoculum, but in this case the entire root system was weighed, cut into pieces with a shears, and macerated in a blender in a stronger solution of NaOC1 (0.25-0.30%) for three periods of 15 sec, separated by two intervals of 10 sec. This extra blending was needed to free eggs and second-stage juveniles embedded in the lignified root tissue. Nematodes were then concentrated using sieves with pore sizes of 0.150, 0.074 and 0.025 mm (100, 200 and 500 mesh, respectively). Root tissue and debris collected on the 100-mesh sieve were discarded. The resistance rating of each rootstock was estimated according to the scale suggested by Taylor and Sasser (23) based on nematode reproduction and root galling: I = immune; HR = highly resistant; R = resistant; MR = moderately resistant; and S = susceptible.

The *P. vulnus* population in the soil was extracted in the same way as were populations of *Meloidogyne* spp. Nematodes in roots, however, were extracted by macerating root tissue in water without NaOC1. Recovery was made with sieves of the same openings used for root-knot nematodes. For *P. vulnus*, a host vs. nonhost rating was established: NH (non host) = Pf < Pi; PH (poor host) = Pi < Pf < 1 500 nematodes per plant; H (host) = Pf > 1 500 nematodes per plant.

Plants were watered daily, or as needed, and fertilized with a full strength Hoagland's nutrient solution once a week. Experiments were conducted in a greenhouse with controlled temperature (20–27 C). Inoculated containers were placed in a sand bed to avoid temperature and humidity fluctuations. In all experiments, each material was replicated seven times in a completely randomized design. Data were analyzed by a one-way analysis of variance. Data for gall index, final nematode population, and nematodes per gram of root data were $\log_{10}$ (N + 1) transformed. Means were compared by Duncan's Multiple Range Test ($P < 0.05$).

To complement the host suitability study of *P. vulnus*, hystological preparations were made to observe the effects of the nematodes in root tissues. Selected root pieces of Garrigues, Garfí, and G × N No 1 were washed free of soil particles, fixed in FAA, dehydrated in a tertiary butyl alcohol series, embedded in a 56 C

Table 2. Reproduction and gall indices of *Meloidogyne incognita, M. arenaria,* and *M. hapla* 4 months after inoculation with 2 000 nematodes per plant in four commonly used almond rootstocks in Spain.

Meloidogyne species	Rootstock or variety	Total population per plant	Nematodes per gram of roots	Gall index (1–6)
M. incognita	Atocha	23 510 ab	1 690	6.0 a
	Garrigues	40 980 ab	1 970	6.0 a
	Marcona	44 540 a	5 130	6.0 a
	D. Largueta	10 040 b	1 760	5.1 b
			NS	
M. arenaria	Atocha	9 070	490	5.3
	Garriguez	21 470	1 390	5.2
	Marcona	6 670	960	4.9
	D. Largueta	17 300	2 760	5.3
		NS	NS	NS
M. hapla	Atocha	17 420	1 620	5.9 a
	Garrigues	14 140	1 080	4.3 b
	Marcona	6 530	1 720	5.7 ab
	D. Largueta	6 920	1 340	5.0 ab
		NS	NS	

[z]Data are means of seven replications. Arithmetic means are presented, but data were transformed to $\log_{10}$ (N + 1) for analysis. Means in columns followed by the same letter do not differ according to Duncan's Multiple Range Test ($P < 0.05$).

paraffin wax, and sectioned in a microtome at 15-18 μm. Sections were stained with safranin and fast green.

RESULTS

In the first experiment, the four almond varieties were susceptible to the three root-knot nematodes species (Table 2). Marcona inoculated with *M. incognita* had a significantly higher final population than Desmayo Largueta. This last variety also differed from the rest in its lower root galling. Garrigues inoculated with *M. hapla* showed a lower gall index in relation to the other materials. Variability of the nematode reproduction parameters was high.

In the second experiment, the almond variety D-3-5 was highly resistant to *M. javanica* (Table 3). The hybrid selection G × N No 9 was resistant, although it did differ significantly from D-3-5. The hybrid Cachirulo was moderately resistant, showing slight galling and a low level of parasitism. Only its final population differed from that of D-3-5 and G × N No 9. The six remaining materials had different degrees of susceptibility to *M. javanica*. The most susceptible was the almond Garrigues, which had a 4.33 galling index, the highest total population in soil and roots (57 020), and the highest number of nematodes per gram of root (3 045).

In the third experiment, all materials tested were good hosts for *P. vulnus* (Table 4). The peach almond-hybrid G × N No 1 presented a significantly higher level of parasitism than the rest. Garrigues was significantly less parasitized than were Cachirulo, Garfí, and G × N No 1. Garrigues also presented the lowest final population, although it did not differ significantly from the majority of the tested materials.

In the histological study with *P. vulnus*, dark necrotic lesions were found throughout the root systems of the almonds Garrigues and Garfí and the peach-almond hybrid G × N No 1. The nematode colonized extensively the cortical parenchyma of the roots forming large pockets that contained all stages of the nematode (Fig. 1A), as well as cavities (Fig. 1B). Migration within the roots was inter and intracellular, and generally

Table 3. Gall index and population levels of *Meloidogyne javanica* in nine *Prunus* rootstocks 4 months after inoculation with 1 000 nematodes per plant.

Rootstocks[y]	Gall index (1-6)	Total population (soil and roots)	Nematodes per gram of root	Resistance rating[z]
D-3-5	1.00 a	0 a	0 a	HR
G × N No 9	2.00 ab	60 a	10 a	R
Cachirulo	2.28 ab	760 b	70 ab	MR
Guara	2.83 ab	5 090 c	600 c	S
Tuono	3.66 bc	9 839 c	920 c	S
Garfí	2.83 ab	13 500 c	870 c	S
T. Verdiere	4.20 c	18 350 c	2 230 c	S
Desmayo Rojo	3.33 abc	19 160 c	2 460 c	S
Garrigues	4.33 c	57 020 c	3 050 c	S

[y]Data are means of seven replications. Arithmetic means are presented, but data were transformed to $\log_{10}$ (N + 1) for analysis. Means in columns followed by the same letter do not differ according to Duncan's Multiple Range Test ($P < 0.05$).

[z]HR = highly resistant; R = resistant; MR = moderately resistant; S = susceptible.

Table 4. Total population and nematodes per gram of roots of *Pratylenchus vulnus* in eight *Prunus* rootstocks at 4 months after inoculation with 1 000 nematodes per plant.

Rootstocks[y]	Total population (soil and roots)	Nematodes per gram of root	Host rating[z]
Garrigues	14 870 a	260 a	H
D-3-5	17 490 a	766 ab	H
Tuono	22 870 a	1 320 ab	H
T. Verdiere	23 200 a	840 ab	H
Guara	32 740 a	1 060 ab	H
Cachirulo	36 050 a	1 960 b	H
Garfí	39 200 a	2 040 b	H
G × N No 1	76 400 b	4 140 c	H

[y]Data are means of seven replications. Arithmetic means are presented, but data were transformed to $\log_{10}$ (N + 1) for analysis. Means in columns followed by the same letter do not differ according to Duncan's Multiple Range Test ($P < 0.05$).
[z]NH = non host; PH = poor host; H = host.

parallel to the stele, although occasionally no defined orientation of the nematode was observed (Fig. 2A). In most cases, nematode eggs were laid inside individual root cells (Fig. 2B). Damaged or ruptured cells along nematode pathways showed heavy discoloration and granulation (brown) as a result of nematode feeding and movement. This was especially evident in the peach-almond hybrid G × N No 1. Eggs, larval, and adult forms were mainly found in healthy tissue or in areas with incipient necrosis. Occasionally, eggs could be found in necrotic tissue. *Pratylenchus vulnus* was not found in the vascular tissues or in the root meristems. No clear histopathological differences were found between the two different almond rootstocks and the peach almond-hybrid.

DISCUSSION

Atocha, Garrigues, and Desmayo Largueta are three of the most widely used almond rootstocks derived from seedlings in Spain. Marcona is the main variety used for fruit production, although occasionally it is used as a rootstock in some regions of Spain, Portugal, and North Africa. Unfortunately, all are quite susceptible to root-knot nematodes and to *P. vulnus* (13).

The most significant result of this study was the detection of different levels of resistance to *M. javanica* in the experimental rootstocks D-3-5, G × N No 9, and Cachirulo. The selection D-3-5 had no visible galls and no nematodes were recovered from soil or roots at the end of the experiment, suggesting that D-3-5 is highly resistant or immune. Bioassay tests to determine penetration, development, and reproduction of *M. javanica* in root tissues are needed to confirm this. D-3-5 is derived from a cross between the almonds Tuono and Titan. Tuono was used for the transmission of desirable self-compatibility characters (21). The hybrid G × N No 9 was found to be highly resistant to *M. incognita* in a previous study (14). This range of resistance to several root-knot species makes G × N No 9 one of the most interesting materials so far tested. Both G × N genotypes are vigorous, and have red leaf, good compatibility with almond varieties, good soil adaptability, and other suitable characters. They derive from crosses be-

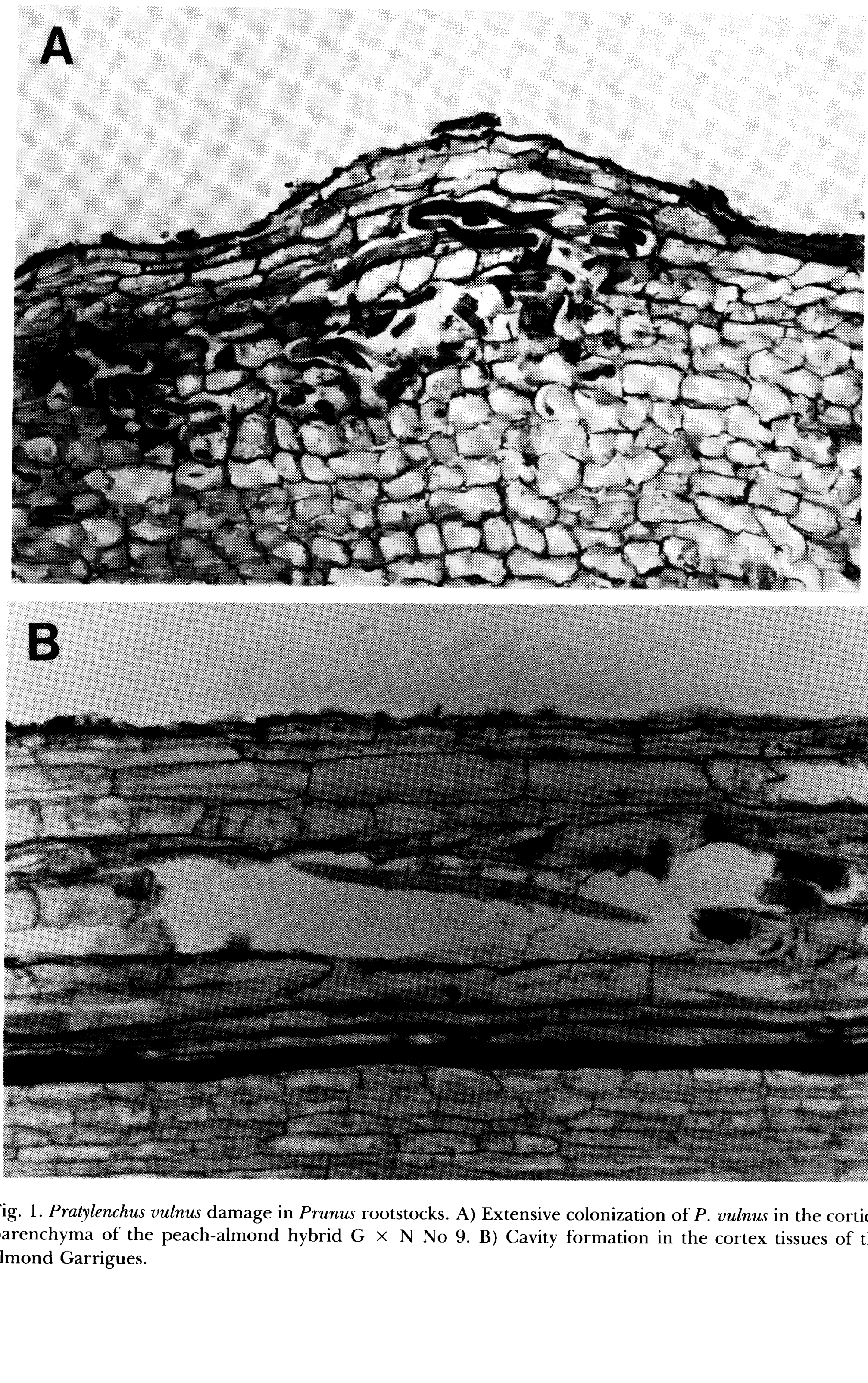
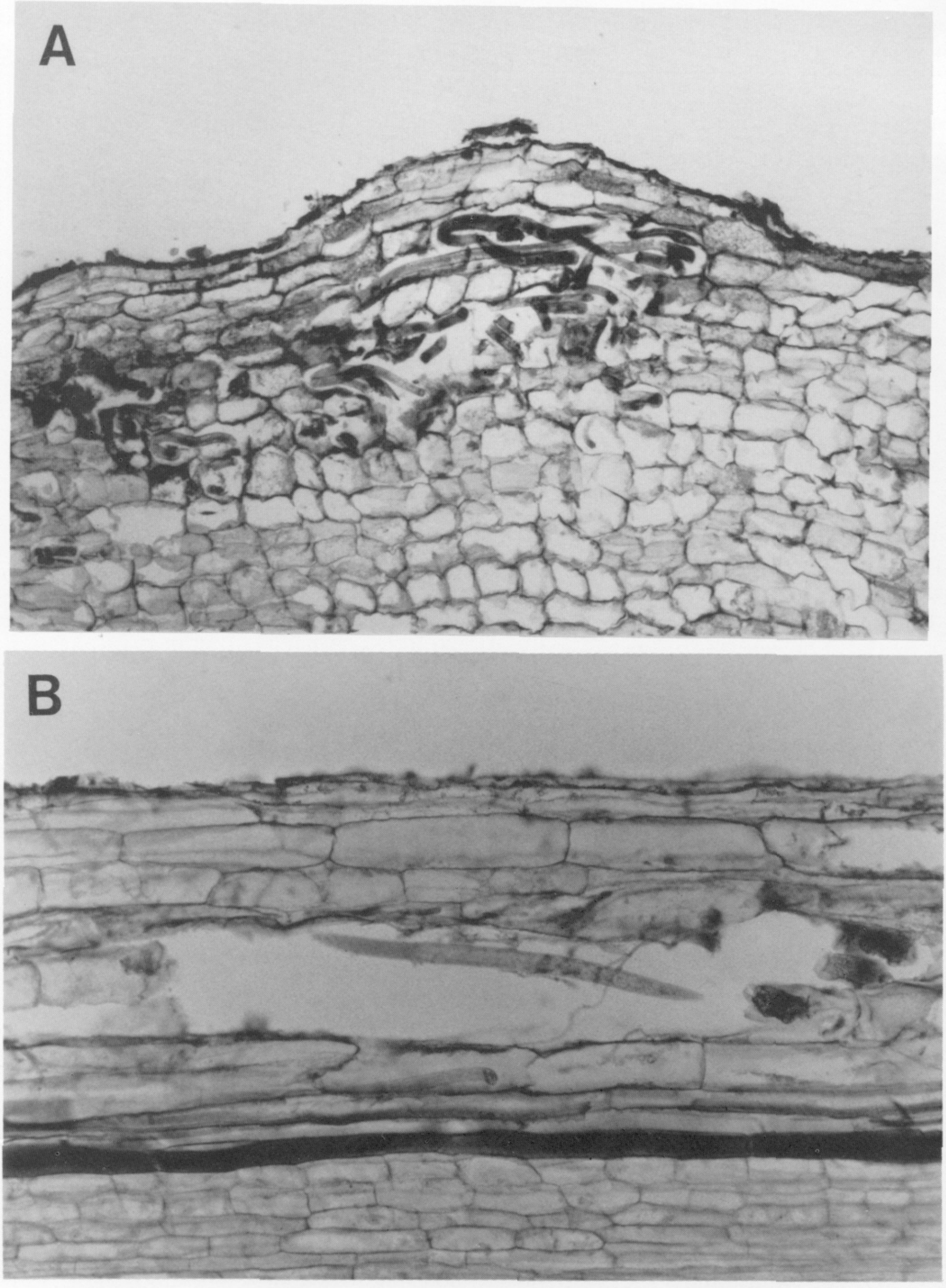


Fig. 1. *Pratylenchus vulnus* damage in *Prunus* rootstocks. A) Extensive colonization of *P. vulnus* in the cortical parenchyma of the peach-almond hybrid G × N No 9. B) Cavity formation in the cortex tissues of the almond Garrigues.



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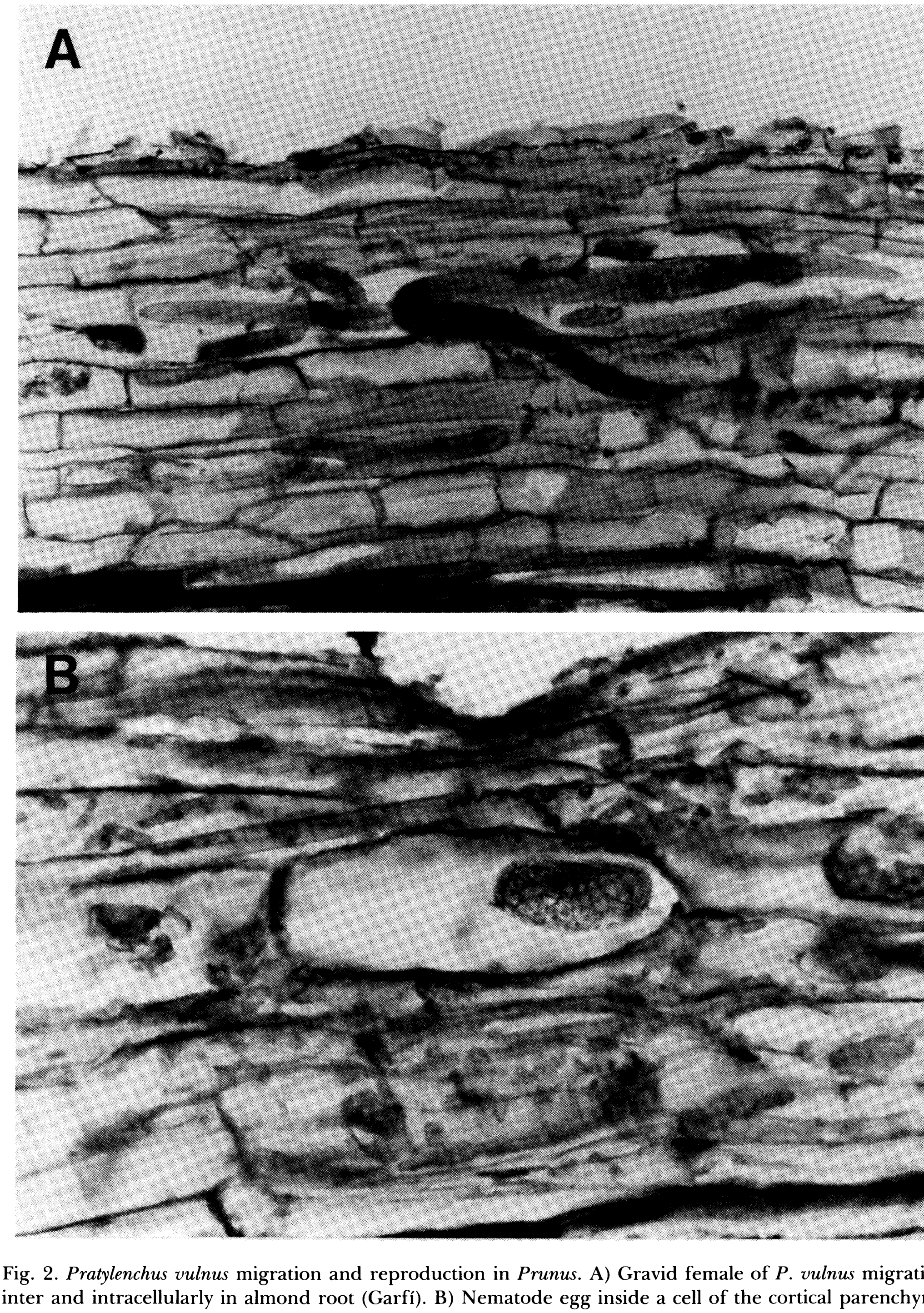
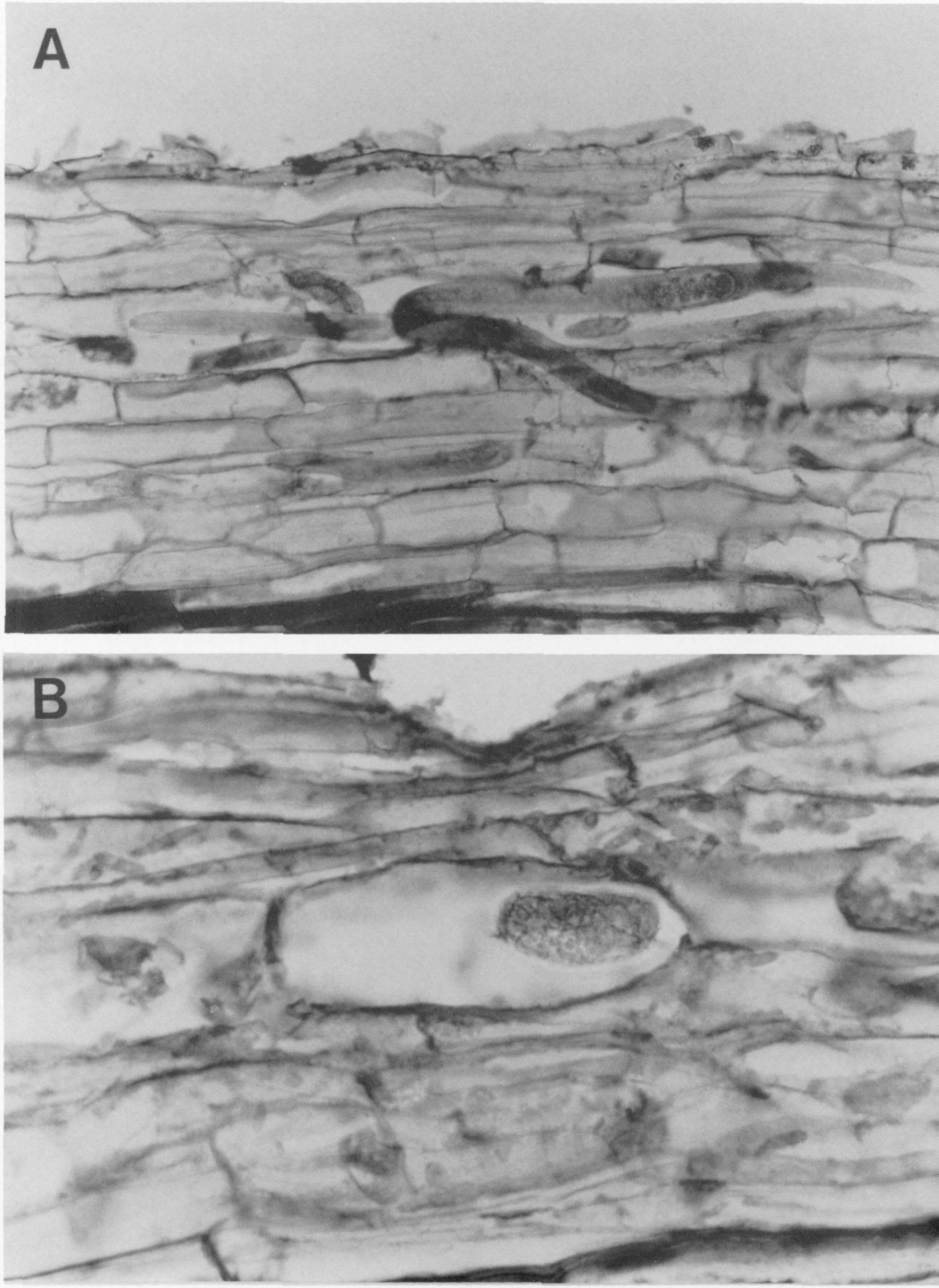


Fig. 2. *Pratylenchus vulnus* migration and reproduction in *Prunus*. A) Gravid female of *P. vulnus* migrating inter and intracellularly in almond root (Garfí). B) Nematode egg inside a cell of the cortical parenchyma of the almond Garrigues.



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tween the almond (female parent) Garfí and the root-knot nematode resistant peach (male parent) Nemared (18). Garfí is a selection of Garrigues. It is vigorous and well adapted to dryland and calcareous soils as is its Garrigues parent, but is significantly less susceptible to root galling caused by *M. javanica*. The high level of resistance in both D-3-5 and G × N No 9 to *M. javanica* suggests that this resistance is dominant and probably determined by a few genes, similar to other cases of root-knot resistance inheritance in peach and almond (4,20,24).

All materials tested against *P. vulnus* were good hosts with varying degrees of parasitism. Unfortunately, the hybrid selection G × N No 1, highly resistant to *M. incognita* and immune to *M. arenaria* (14), was significantly more parasitized than the rest of the materials, with final populations reaching 4 140 nematodes per gram of root and 76 400 total nematodes in soil and roots in 4 months (Table 4). On the other hand, Garfí was a significantly better host for *P. vulnus* than was its parent, Garrigues (2 040 vs. 260 nematodes per gram of root, respectively), which could explain in part the higher population increase in its progeny, G × N No 1. The level of tolerance of G × N No 1 to *P. vulnus* is unknown. If the rapid population increase that we observed in pots also occurs in orchards, a high level of tolerance would be required to escape economic injury in orchards where *P. vulnus* is present.

The histopathology of *P. vulnus* on almond and peach-almond hybrid is quite similar to that which occurs in other woody hosts (5), except that in this study it was not seen invading or feeding on the vascular tissues. The extensive colonization and destruction of the cortical parenchyma of the root would suggest that this nematode is likely to cause economic damage, especially in young infested plants.

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