HOST-PARASITE RELATIONSHIP OF *PRATYLENCHUS VULNUS* ON APPLE AND PEAR ROOTSTOCKS

C. Fernández, J. Pinochet, and R. Dolcet

Departamento de Patología Vegetal y Departamento de Genética, Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Ctra. de Cabrils s/n, 08348 Cabrils, Barcelona, España.

ABSTRACT

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The effects of *P. vulnus* on the apple rootstock EMLA 26 and the pear rootstock OHF-333 were evaluated *in vitro* and under greenhouse conditions. *In vitro*, *P. vulnus* caused extensive damage to both rootstocks in the absence of microorganisms. Earliest penetration was recorded at 2 days for both hosts. Nematodes appeared to converge to one or two points of entry. During the course of 28 days, cortical parenchyma became extensively colonized and developed lesions and cavities. *Pratylenchus vulnus* was randomly oriented within the cortex and did not invade meristems or conductive tissues. Visible lesions appeared at 7 days and expanded rapidly. In greenhouse experiments, shoot length, and fresh top and root weights in 5-month-old apple and pear were significantly lower in *P. vulnus* treatments than in uninoculated controls. Plant growth of pear did not differ between treatments receiving 50 and 500 nematodes/plant. The EMLA 26 rootstock was more susceptible to *P. vulnus* than the OHF-333 pear rootstock.

Key words: apple, host-parasite relationship, *Malus silvestris*, pear, *Pratylenchus vulnus*, *Pyrus communis*, rootstock.

RESUMEN

Fernández, C., J. Pinochet y R. Dolcet. 1992. Relación parásito hospedador de *Pratylenchus vulnus* en patrones de manzano y peral. Nematrópica 22:227–236.

Los efectos de *P. vulnus* fueron evaluados en el patrón de manzano EMLA 26 y en el patrón de peral OHF-333 bajo condiciones *in vitro* e invernadero. En las pruebas *in vitro*, *P. vulnus* causó un daño extensivo en ambos patrones en ausencia de microrganismos. La penetración más temprana se registró a los 2 días para ambos hospedadores. Los nematodos parecen converger hacia uno o dos puntos de penetración. Durante 28 días de infección, el parénquima cortical fue colonizado extensivamente con formación de cavidades. *Pratylenchus vulnus* no mostró ninguna orientación definida dentro del parénquima cortical. El nematodo no se encontró en los meristemas ni tejidos de conducción. Lesiones visibles aparecieron a los 7 días, las cuales se expandieron con rapidez. La longitud del tallo, peso aéreo y peso radicular en manzano y peral fueron significativamente más bajos en tratamiento con *P. vulnus* en comparación con el control sin nematodos. No hubo diferencias en el crecimiento de los perales entre tratamientos inoculados con 50 y 500 nematodos/planta. El patrón de manzano EMLA 26 fue más susceptible a *P. vulnus* que el peral OHF-333.

Palabras clave: Malus silvestris, manzano, patrón, peral, *Pratylenchus vulnus*, *Pyrus communis*, relación parásito-hospedador.

INTRODUCTION

The most important root-lesion nematode found in fruit tree orchards and nurseries in the Mediterranean area is *Pratylenchus vulnus* Allen & Jensen (6,9, 22). This species is considered an important pathogen in several stone fruit and nut tree crops throughout the world (4, 13). In Spain, it has been detected in rose (*Rosa multiflora* L.), apple (*Malus silvestris* L.), pear (*Pyrus communis* L.), almond (*Prunus amygdalus* Batsch), peach (*Prunus persica* Stock.), and quince (*Cydonia oblonga* Miller). Its geographical distribution in Spain has not been examined.

The extent of economic damage caused by *P. vulnus* on apple is unknown. However, it is suspected to be similar to that of *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans Stekhoven, another root-lesion nematode that is frequently found attacking apple (1,11,19) and peach (15) in the northeastern United States and in parts of Europe (3) where cooler climate prevails. Disease severity in apple seedlings a few weeks old inoculated with *P. penetrans* seems to be considerable (7). In warmer mediterranean environments where calcareous soils are common, *P. penetrans* is less frequent on fruit tree crops (22) and appears to be replaced by *P. vulnus*. Recent host range studies conducted in Spain have shown the apple rootstocks EMLA 9 and EMLA 106, and pear rootstock OHF-333 to be good hosts of *P. vulnus* (18). These rootstocks together with EMLA 26 apple have been introduced into the Spanish market in recent years and have become quite popular among growers. The purposes of this study were to examine the host-parasite relationship between *P. vulnus* and rootstocks of apple and pear in the absence of microorganisms, and to determine the damage of *P. vulnus* to young plantlets under greenhouse and shadehouse conditions.

MATERIALS AND METHODS

Apple rootstock EMLA-26 and pear rootstock OHF-333 were obtained from Agromillora Catalana S.A., Sant Sadurní d'Anoia, Barcelona, Spain. Plant material was micropropagated.

The *P. vulnus* population used was isolated from rose in Cabrils, Barcelona, and cultured monoxenically on carrot disks (14). Identification to species level was made by the Commonwealth Institute of Parasitology, St. Albans, United Kingdom. All *P. vulnus* inoculum used in experiments was obtained from carrot disk cultures by adding sterile water to the cultures and collecting the nematodes with a pipette.

In vitro tests: Both apple and pear rootstocks were micropropagated axenically on nutrient agar (16) in 21-mm-diam culture tubes. Nematodes from carrot disk cultures were surface sterilized with 2 000 ppm streptomycin sulfate and rinsed twice in sterile water prior to inoculation. Approximately 0.2 ml of nematode suspension containing 100 individuals of mixed larval and adult stages were pipetted onto the surface of the agar in each culture tube. Inoculated plantlets were of uniform size and were maintained at 21–23 C in an illuminated growth chamber. Plantlets were harvested in sets of four plants at 2, 7, 14, 21, and 28 days after inoculation. At each harvest date, tissue invasion and nematode reproduction were evaluated by washing roots free of nutrient medium and staining whole root systems with lactophenol and acid fuschin. Roots were then cleared in lactophenol-glycerine in an oven at 65 C for 24 hr. The root system of each plantlet was cut into 1-cm pieces, that were sliced in half longitudinally and mounted on a microscope slide. The nematodes of each developmental stage inside the roots were counted separately on each harvest date. Roots containing too many nematodes to count were cut into thinner slices and mounted again. Plantlets of both *in vitro* experiments were arranged in a completely randomized design. A separate set of inoculated apple and pear plants were collected for histological examination by light microscopy and scanning electron microscopy (SEM). For light microscopy, selected root pieces of EMLA-26 and OHF-333 were fixed in FAA, dehydrated

in a tertiary butyl alcohol series, embedded in a paraffin wax at 56 C and sectioned with a microtome at 15–18 μm. Sections were stained with safranin and fast green. For SEM observations, cellular contents of root tissue were digested, then root pieces were dehydrated to critical point in CO_2, mounted, coated with gold (2), and examined at 15 kV.

Greenhouse and shadehouse experiments: The pathogenicity of *P. vulnus* to apple and pear were evaluated separately in two similar experiments. In both experiments, plantlets that had been micropropagated in agar were transferred to 600-ml pots with a 8:1 (v:v) quartz-sand peat mixture, and climatized in a high humidity chamber for 20 days. Plants were then transferred to 2.4-L pots that contained a loamy sand textured soil (78% sand, 20% silt, 2% clay, pH 7.12, < 4% organic matter). Nematode suspensions recovered from stock cultures as previously described were adjusted to deliver 50 and 500 nematodes/plant through four holes 4–5 cm from the base of the stem. Uninoculated plants of both rootstocks were included as controls. Pots were placed in a sand bed in a greenhouse to minimize temperature and humidity fluctuations. Ambient temperature in the greenhouse was 17–26 C. In the early summer (after 10 weeks in the apple experiment and after 14 weeks in the pear experiment), pots were transferred to a shadehouse (16–31 C) where they were maintained for 2 more months. Plants were watered as needed and fertilized with full strength Hoagland's nutrient solution (5) once a week. In each experiment, treatments were replicated eight times in a completely randomized design. Nematode reproduction and plant growth (top weight, root weight, shoot diameter, and shoot length) were assessed 135 days and 165 days after inoculation in the apple and pear experiments, respectively. Nematodes in soil were recovered by differential sieving and sugar flotation (8) from a 250-cm^3 subsample taken from each pot after thoroughly mixing the soil. Nematodes in roots were extracted by cutting the whole root system into pieces *ca.* 1-cm long and macerating them in water with a commercial blender for 30 s in three 10-s intervals. The resulting nematode suspension was cleaned up and concentrated by two passes through nested 0.150-mm and 0.025-mm sieves (100 and 500 mesh, respectively). Root tissue and debris collected on the 0.150-mm sieve were discarded.

Data were analyzed by a one-way analysis of variance. Means for top weight, root weight, shoot diameter, shoot length, final nematode population, and nematodes per gram of root were compared by Tukey's multiple range test ($P \leq 0.05$).

RESULTS

In vitro *experiments:* Under monoxenic conditions, *P. vulnus* caused extensive damage to apple and pear rootstocks. The first signs of root discoloration were recorded at 7 days for both apple and pear. Lesions could be seen through the glass tube and growth medium after 14 days. Earliest tissue penetration was recorded at 2 days for both hosts. On apple, most nematodes used as inoculum were found aggregated in one portion of the root, indicating that they had converged to one point following initial penetration by one or several nematodes. Nematodes reproduced rapidly thereafter reaching 7.7-fold at 14 days and 26.4-fold at 28 days on apple (Table 1). On pear, population buildup was lower than on apple. The highest densities were recorded at

Table 1. Total numbers of *Pratylenchus vulnus* found within roots of monoxenically micropropagated plantlets of EMLA 26 apple 2, 7, 14, 21, and 28 days after inoculation with 100 nematodes/plant.

Days after inoculation	Eggs	Larvae	Males	Females	Total[z]
2	0	31	15	34	80±79
7	158	25	17	55	255±204
14	558	60	41	110	769±510
21	472	88	24	80	664±519
28	2 183	242	64	145	2 634±447

Mean of four plants per inoculation date.
[z]Total population in roots ± standard deviation.

21 days, reaching 4-fold on pear (Table 2). On both hosts, the number of eggs outnumbered the rest of the stages from 7 to 28 days. A lower male/female ratio was observed on pear. On pear, one replication for each harvest date was lost due to contamination.

In tissue prepared for histological examination by SEM, all plantlets infected with *P. vulnus* showed obvious lesions in young, actively growing roots. Following tissue penetration, *P. vulnus* migrated intercellularly, extensively colonizing the tissues of the cortical parenchyma (Fig. 1A). Cross-sections of apple feeder roots revealed that the inner layers of cortical cells close to the endodermis had collapsed producing cylindroid cavities detached from the stele (Fig. 1B). Roots contained all stages of the nematode. Thin sections examined by light microscopy showed cavity formation with clear destruction of cell walls (Fig. 2A). Patterns of cell destruction resulting from nematode migration indicated no consistent orientation of *P. vulnus* within the cortical tissues. Frequently, larval and adult stages were found coiled or oriented circumferentially to the root axis (Fig. 2B). Cells adjacent to nematode pathways, cavities, and feeding sites had dense cytoplasmatic granulation and stained in contrasting red and brownish tones (Fig. 2C). Collapsed tissue and the destruction of cell walls gave the cortical parenchyma a deformed appearance and cells were of irregular sizes and shapes when compared to uninfected parenchyma tissue. All developmental stages were found both in tissue with incipient

Table 2. Total numbers of *Pratylenchus vulnus* found within roots of monoxenically micropropagated plantlets of OHF-333 pear 2, 7, 14, 21, and 28 days after inoculation with 100 nematodes/plant.

Days after inoculation	Eggs	Larvae	Males	Females	Total[z]
2	3	16	6	18	43±5
7	40	23	6	28	97±58
14	226	69	36	70	401±177
21	232	78	8	85	405±143
28	136	44	12	39	231±196

Mean of three plants per inoculation date.
[z]Total population in roots ± standard deviation.

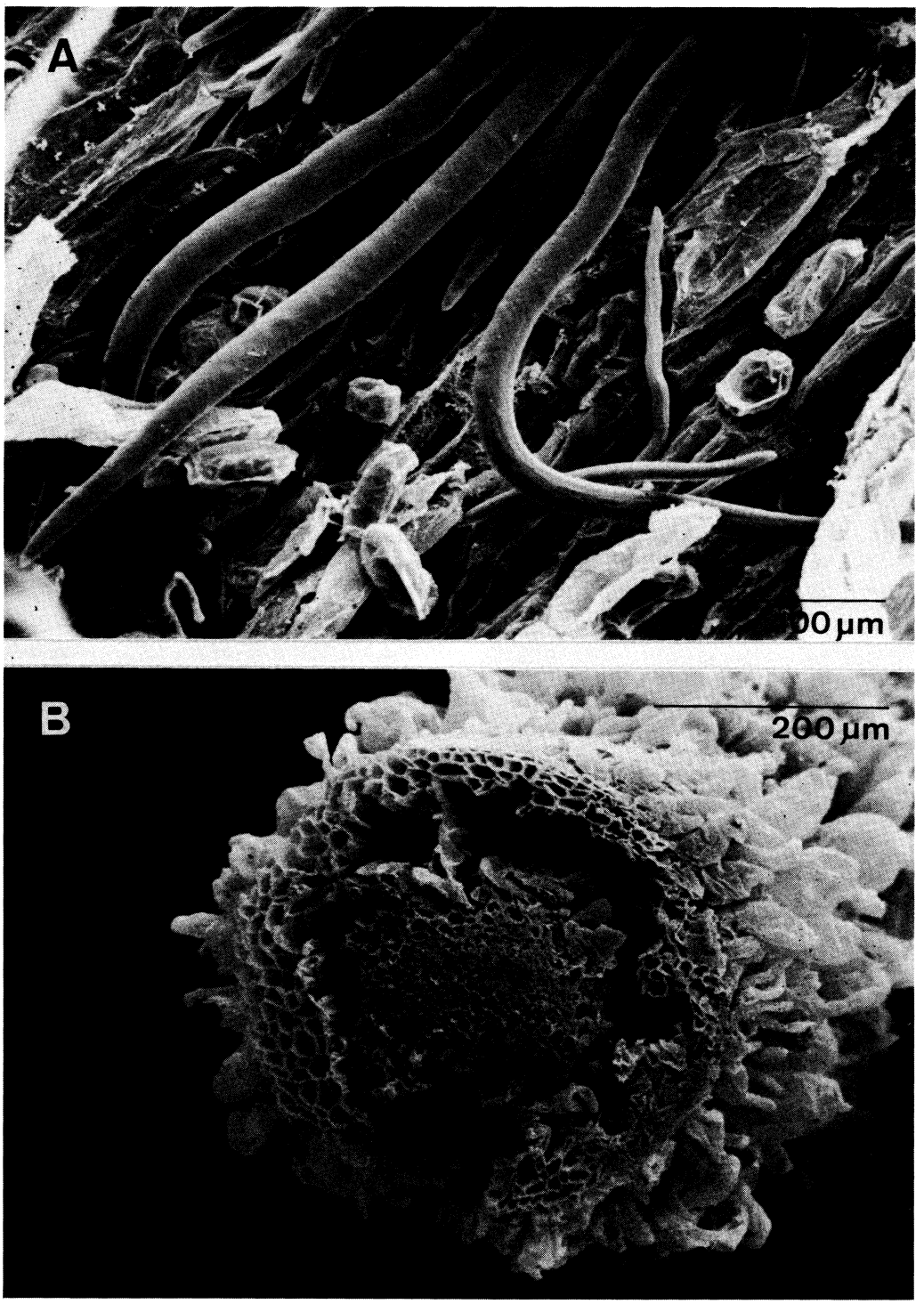
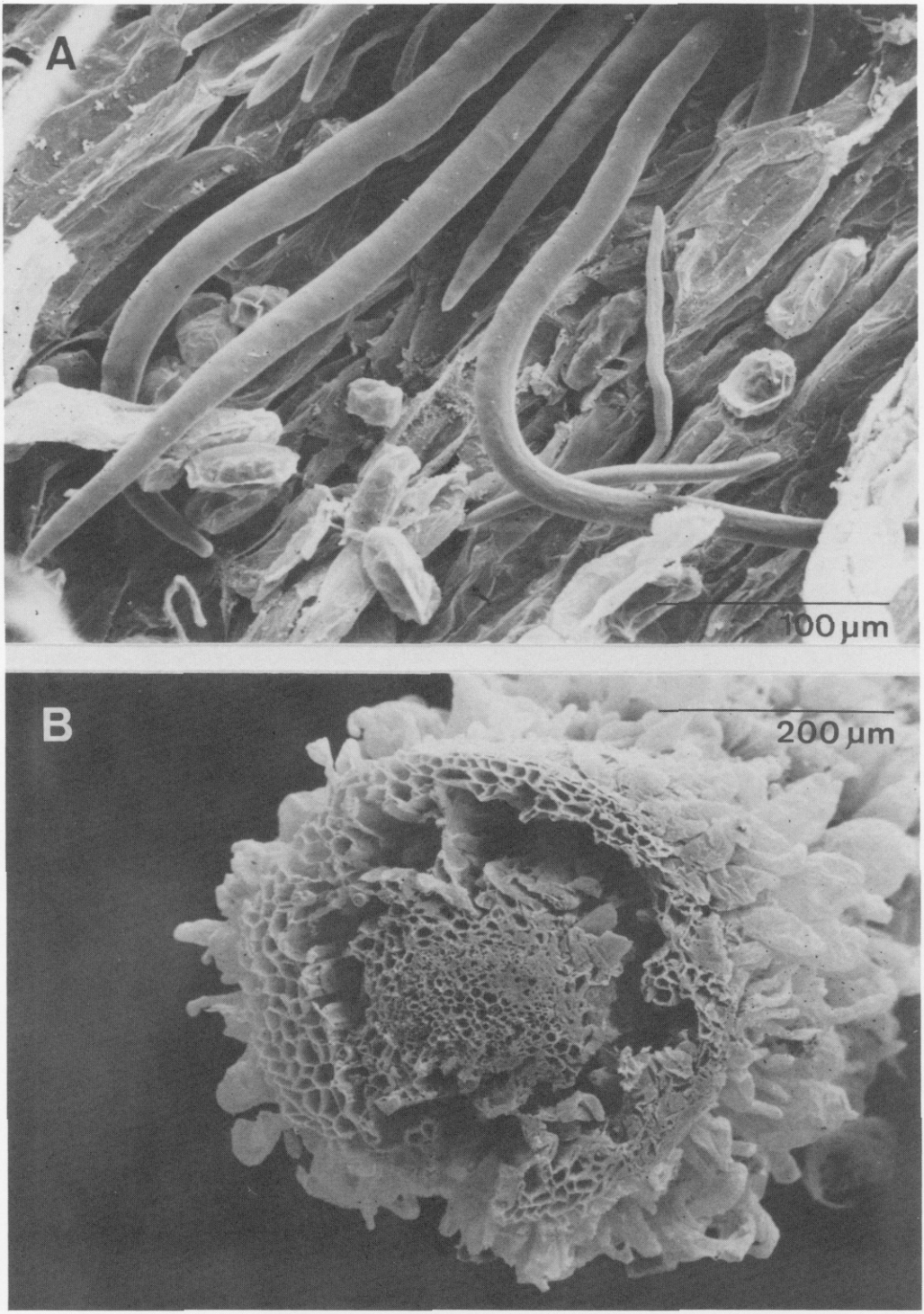


Fig. 1. Roots of apple rootstock EMLA 26 infected with *Pratylenchus vulnus*. A) Extensive colonization of the cortical parenchyma by adults, larvae, and eggs. B) Cross-section of root with the cortical parenchyma collapsed and partially detached from the central cylinder.



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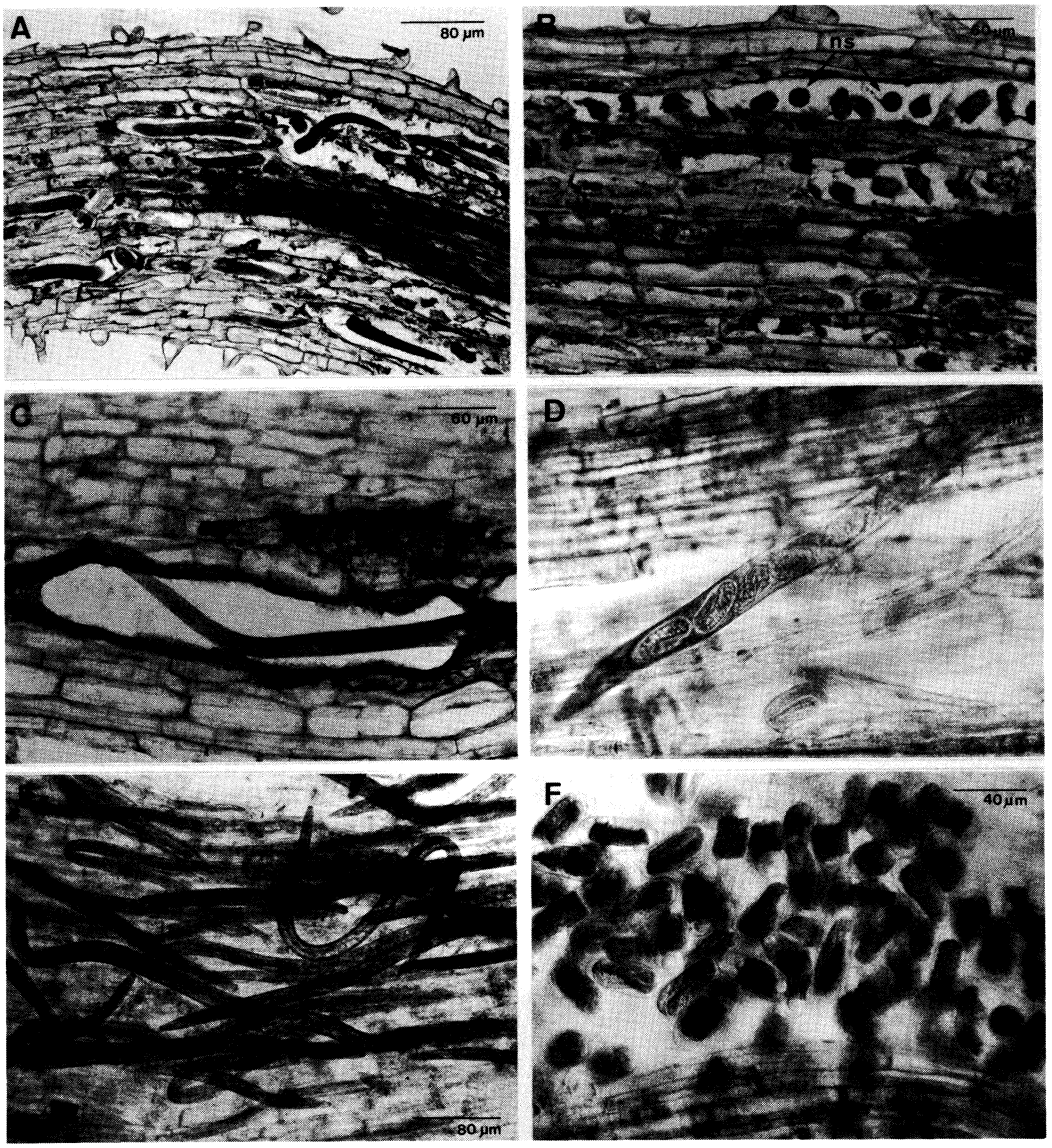

Fig. 2. Damage and reproduction of *Pratylenchus vulnus* on OHF-333 pear and EMLA 26 apple rootstocks. A) Nematode damage to the cortical parenchyma of OHF-333. B) Sections of larval and adult stages showing a perpendicular orientation to the conductive tissues in OHF-333 (ns = nematode sections). C) Adult inside a cavity showing destruction of cell walls and discoloration of adjacent tissue on EMLA 26 apple. D) Female of *P. vulnus* containing six eggs. E) Initial inoculum aggregating in one portion of the root 2 days after inoculation with 100 *P. vulnus* on EMLA 26. F) Sections of the cortical parenchyma packed with eggs 28 days after inoculation on EMLA 26.

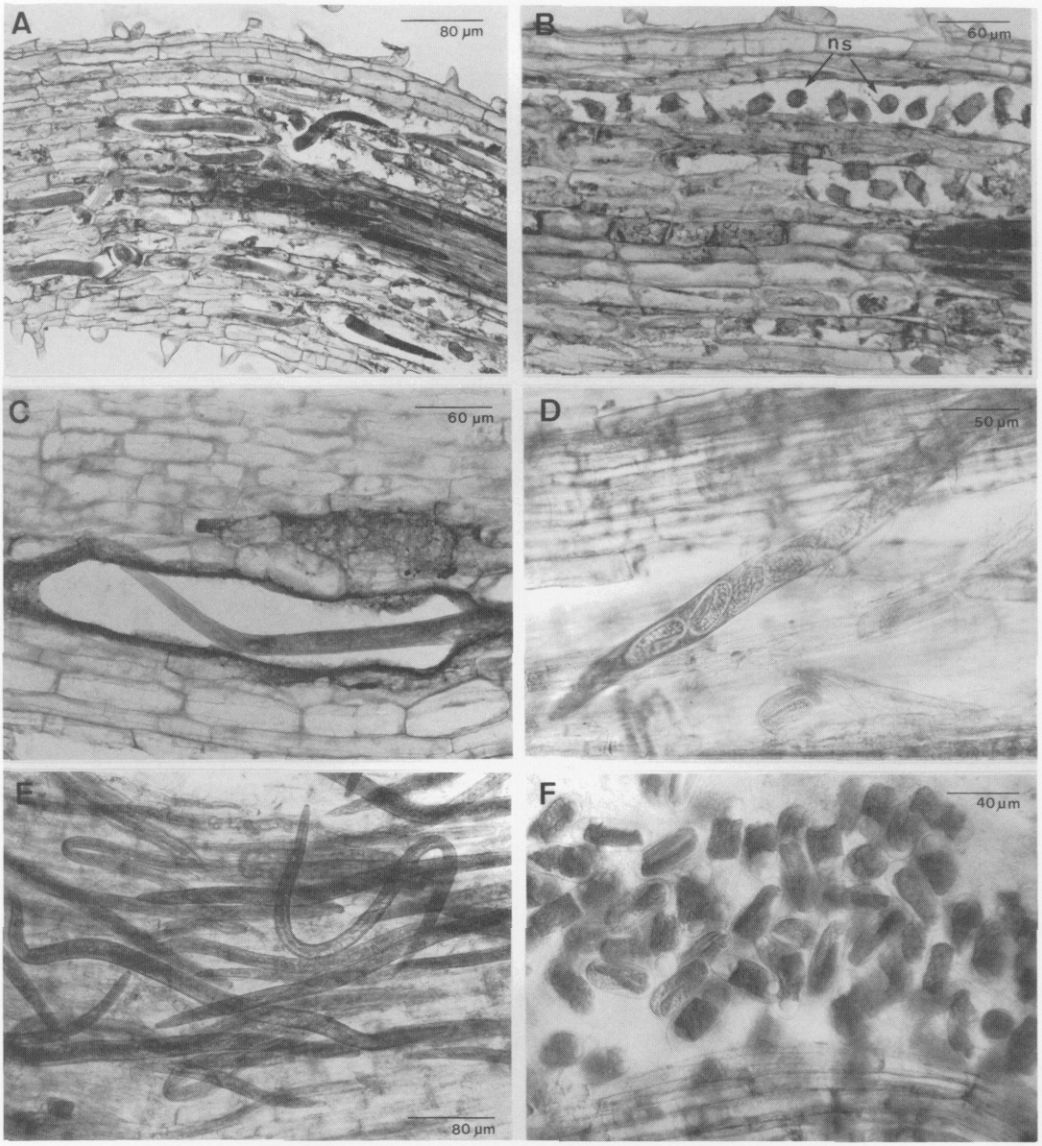

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Table 3. Growth of apple rootstock EMLA 26 and reproduction of *Pratylenchus vulnus* in a greenhouse/shadehouse experiment 135 days after inoculation with 50 nematodes/plant.

Treatment	Fresh top weight (g)	Shoot diameter (mm)	Shoot length (cm)	Fresh root weight (g)	Final nematode population[y]	Nematodes per gram of root	Pf/Pi[z]
Control	5.14 a	4.37 a	16.42 a	6.01 a	—	—	—
50 *P. vulnus*	1.76 b	3.92 a	5.33 b	2.77 b	83 600	18 693	1 672

Data are means of eight replications. Means in columns for each rootstock followed by the same letter do not differ according to Tukey's multiple range test ($P \leq 0.05$).
[y]Nematodes in roots and soil.
[z]Pf/Pi = Final population/Initial population.

necrosis and in apparently healthy tissue. Many females were found with two eggs inside the ovary; one female contained six eggs (Fig. 2D). At 28 days, sections of the cortical parenchyma were packed with eggs (Fig. 2F). Nematodes were not detected inside meristematic or vascular tissues, nor were they found feeding on or penetrating cells of the endodermis. No obvious histopathological differences were noted between the apple EMLA 26 and pear OHF-333 rootstocks.

Greenhouse and shadehouse experiments: Shoot length and the fresh top and root weights of apple EMLA 26 were significantly lower ($P \leq 0.05$) in the *P. vulnus* treatment inoculated with 50 nematodes/plant than in the uninoculated control (Table 3). There were no differences between the two treatments in shoot diameter. Six of the eight replications in the treatment inoculated with 500 nematodes died before harvest. The average top and root weights of the two surviving EMLA 26 replications were 1.89 and 1.87 g, respectively (data for this treatment not presented). On the OHF-333 pear rootstock, shoot length, shoot diameter, and fresh top and root weights in both inoculated treatments were significantly lower ($P \leq 0.05$) than in the uninoculated control. There were no differences between effects of the two inoculum rates on plant development of OHF-333 (Table 4). The highest Pf/Pi values (ratio of final to initial population density) were recorded on apple (1 672) and pear (693) inoculated with 50 nematodes/plant, respectively.

DISCUSSION

In general, the host-parasite relationship of *P. vulnus* on apple appears to be similar (with minor differences) to that

Table 4. Growth of pear rootstock OHF-333 and reproduction of *Pratylenchus vulnus* in a greenhouse/shadehouse experiment 165 days after inoculation with 50 and 500 nematodes/plant.

Treatment	Fresh top weight (g)	Shoot diameter (mm)	Shoot length (cm)	Fresh root weight (g)	Final nematode population[y]	Nematodes per gram of root	Pf/Pi[z]
Control	3.62 a	4.26 a	18.32 a	2.99 a	—	—	—
50 *P. vulnus*	1.34 b	3.17 b	7.41 b	1.17 b	34 653 a	12 865 a	693
500 *P. vulnus*	0.89 b	2.58 b	5.04 b	0.63 b	62 088 a	7 585 a	124

Data are means of eight replications. Means in columns for each rootstock followed by the same letter do not differ according to Tukey's multiple range test ($P \leq 0.05$).
[y]Nematodes in roots and soil.
[z]Pf/Pi = Final population/Initial population.

described by Pitcher *et al.* (19) for *P. penetrans* on this host.

The *in vitro* tests confirmed previous observations that *P. vulnus* is a virulent pathogen on EMLA 26 apple and OHF-333 pear capable of reproducing quickly and destroying small plantlets in a short period of time. The greenhouse experiments indicated that EMLA 26 apple rootstock is more susceptible than OHF-333 pear since densities of 500 nematodes/plant were capable of killing young apple but not pear plantlets within 80 days of inoculation.

Lesions in the root were not found on apple or pear 2 days after inoculation. In both hosts, the earliest detection of lesions on a dissecting microscope was recorded at 7 days. Incipient lesions were more evident on apple and had a light tan discoloration with redish tones. Presence of the nematode in discolored areas was confirmed by cutting root pieces into 1-cm portions and staining them. In a similar host-parasite relationship study, Pitcher *et al.* (19) found that *P. penetrans* was capable of causing a discoloration of the endodermis and hypodermis 24 hr after inoculation of apple seedlings. In our study, root discoloration could have taken place earlier than the seventh day without being noticed because it was difficult to see through the glass tube and nutrient medium in which plants were grown.

Earliest tissue penetration was recorded at 2 days in both hosts but it is likely that it occurred in the first hours following inoculation because at 2 days most inoculum was found inside the root. The aggregation of these nematodes within one region of the tissue suggests that attraction mechanisms are involved following the first penetration of root tissue (Fig. 2E). A similar phenomenom has been described by Radewald *et al.* (20) for *P. coffeae* (Zimmerman) Filipjèv & Schuurmans Stekhoven in rough lemon (*Citrus jambhiri* Lush.).

The final nematode population on OHF-333 at 28 days was lower (231 nematodes) than at 14 and 21 days (401 and 405 nematodes/plant, respectively). This suggests that maximum buildup could have taken place before the fourth week. The collapse of plant tissue and necrosis might have favoured migration of the root population into the agar medium after the third week. In contrast, population increase on EMLA 26 apple continued progressively after the 21 days reaching 2 634 nematodes/plant at 28 days. The more vigorous root system of apple grown in artificial medium could explain this difference.

In the greenhouse evaluation, *P. vulnus* reached 18 690 nematodes/g of root on apple and 12 870 nematodes/g of root on pear about 5 months after inoculation with 50 nematodes/plant (Tables 3 and 4). This level of reproduction is extremely high compared to results obtained in other host range and pathogenicity studies with this nematode (10,12,21), indicating that apple and pear together with walnut, peach, and cherry are some of the most suitable hosts for *P. vulnus* among fruit and nut tree crops. It is noteworthy that in a previous host suitability study lasting 15 months, *P. vulnus* caused a significant reduction in fresh root weights of OHF-333 but not in top weights when compared to uninoculated plants (17). In this study, all growth parameters (top weight, shoot diameter, shoot length, and root weight) were severely reduced ($P \leq 0.05$) on OHF-333, suggesting that the type of material used previously (hardwood cuttings) and the delay in inoculation after rooting (12 weeks) resulted in tolerance to the nematode, even though inoculation levels

were relatively high (2 000 nematodes/ plant).

From a practical point of view, the damage caused by this migratory endoparasitic nematode can be substantial on apple and pear when plant material is propagated in contaminated nurseries. Substantial damage could also result from transplanting clean but young and vulnerable plants to infested fields, even though initial populations of *P. vulnus* are relatively low.

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