Effects of Pratylenchus vulnus on the Growth of Sour Orange

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Abstract: Pratylenchus vulnus suppressed the growth of sour orange seedlings in greenhouse experiments. Growth retardation (in height, in trunk diameter, and in dry top and root weights) was observed in inoculated plants growing in two soil types. Population density, 13 months after inoculation, averaged more than 1,000 specimens/gm of fresh root. Anatomical studies showed that *P. vulnus* prefers to attack the cortex and causes cavities among the cortical cells. *Key Words:* lesion nematode, population density, soil type.

Pratylenchus spp. are reported as being pathogenic to citrus in greenhouse experiments and as having a wide host range among citrus species (3, 7, 8). *Pratylenchus coffeae* (Zimmermann) Filipj. & Schuur.-Stekh. causes citrus decline in Florida (10), Japan (15), and India (12). Stunting, dieback, and poor growth with feeder-root lesions are the symptoms observed in *P. coffeae*-infected citrus trees. *Pratylenchus vulnus* Allen & Jensen has been associated occasionally with citrus in California (1, 2, 13) and with declining sour orange seedlings in nurseries in Italy (5).

No information is available on the pathogenicity of this species to *Citrus*. This paper reports the pathogenic effects of *P. vulnus* to sour orange (*Citrus aurantium* L.) under greenhouse conditions.

METHODS AND MATERIALS

Two soils, a river sand and Massafra sandy clay loam, were used in this study conducted in Italy. Each soil had previously been steam sterilized and stored for 40 days. Physical and chemical properties of the soil are given in Table 1.

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TABLE 1. Physical and chemical characteristics of soils utilized.

Soil type	pH	Clay (<0.002 mm) (%)	Silt (0.002-0.05 mm) (%)	Sand (0.05-2 mm) (%)	Gravel (>2 mm) (%)	Organic matter (%)
Massafra sandy clay loam	7.60	22.62	10.76	62.20	4.42	1.70
River sand	8.90	0.72	4.11	65.89	29.28	0.02

TABLE 2. Influence of *Pratylenchus vulnus* on the growth of sour orange seedlings 13 months after inoculation.

Soil type and *P. vulnus* initial inoculum level	Total height (cm)	Trunk diam. (mm)	Dry root weight (gm)	Dry top weight (gm)
Sandy clay loam[y]				
100	93.9a	8.1a	11.6a	16.7ab
250	81.4a	7.2b	9.7a	11.1a
500	78.0a	7.8ab	11.5a	13.7a
Control	126.8b	9.2c	15.1b	21.3b
River sand[z]				
100		6.8	8.8	
Control		8.9*	20.9**	

[y]Means followed by the same letters are not significantly different ($P \leq 0.05$) according to Duncan's Multiple Range Test.
[z]Asterisks (*, **) indicate significantly greater than inoculated plants at $P = 5$ and 1% level, respectively.

One-year-old sour orange seedlings were selected for uniformity and transplanted into 18-cm clay pots, each containing one of the soils. Plants growing in Massafra sandy clay loam were inoculated 1 month later by pipetting water suspensions of 100, 250, or 500 *P. vulnus* into four holes spaced evenly in the soil around the base of each plant. Each inoculum level was replicated 10 times, one seedling/replicate. Five replicates of plants growing in the river sand received 100 nematodes/plant 1 month after being transplanted with the same inoculation procedure. Noninoculated plants served as controls. Nematode inoculum was obtained from infected seedling roots collected in a citrus nursery by using the Young's jar incubation method (16). Pots were randomized on a glasshouse bench and plants were grown for 13 months at 24-26 C.

TABLE 3. Mean numbers of *Pratylenchus vulnus*/gm of sour orange fresh root.

Soil type and *P. vulnus* initial inoculum level	No. nematodes (months after inoculation) 6	11	13
Sandy clay loam[y]			
100	230	1,745	1,167
250	305	901	1,185
500	614	1,733	1,313
River sand[z]			
100	3,719	1,145	1,959

[y]Mean of 10 replicates.
[z]Mean of 5 replicates.

Root samples were collected for nematode population counts and plant height measurements were taken at 6, 11, and 13 months after inoculation (always from the same pots). Individual root samples were cut into segments, washed in tap water, and incubated moist in half-liter jars in the dark at 22-24 C (16). Nematodes that emerged were counted at 2 and 4 days. At harvest, top and root oven dry weights and stem diameter measurements were taken. Results were analyzed by either Duncan's multiple range test or Student's 't' test.

So that roots could be examined microscopically, root segments were washed free of soil, fixed in FAA (formalin-acetic acid-alcohol) for 48 h, dehydrated in tertiary butyl alcohol, embedded in paraffin, sectioned at 10-15 μm, stained in safranin-fast green, mounted in Permount, and examined with a compound microscope (6). For examination with a scanning electron microscope, longitudinal and cross sections of roots were cut at 20 μm, washed twice in benzene to remove paraffin, washed in acetone, metalized with gold, and examined (4).

RESULTS AND DISCUSSION

Growth of seedling roots infected with *P. vulnus* in the sandy clay loam soil was

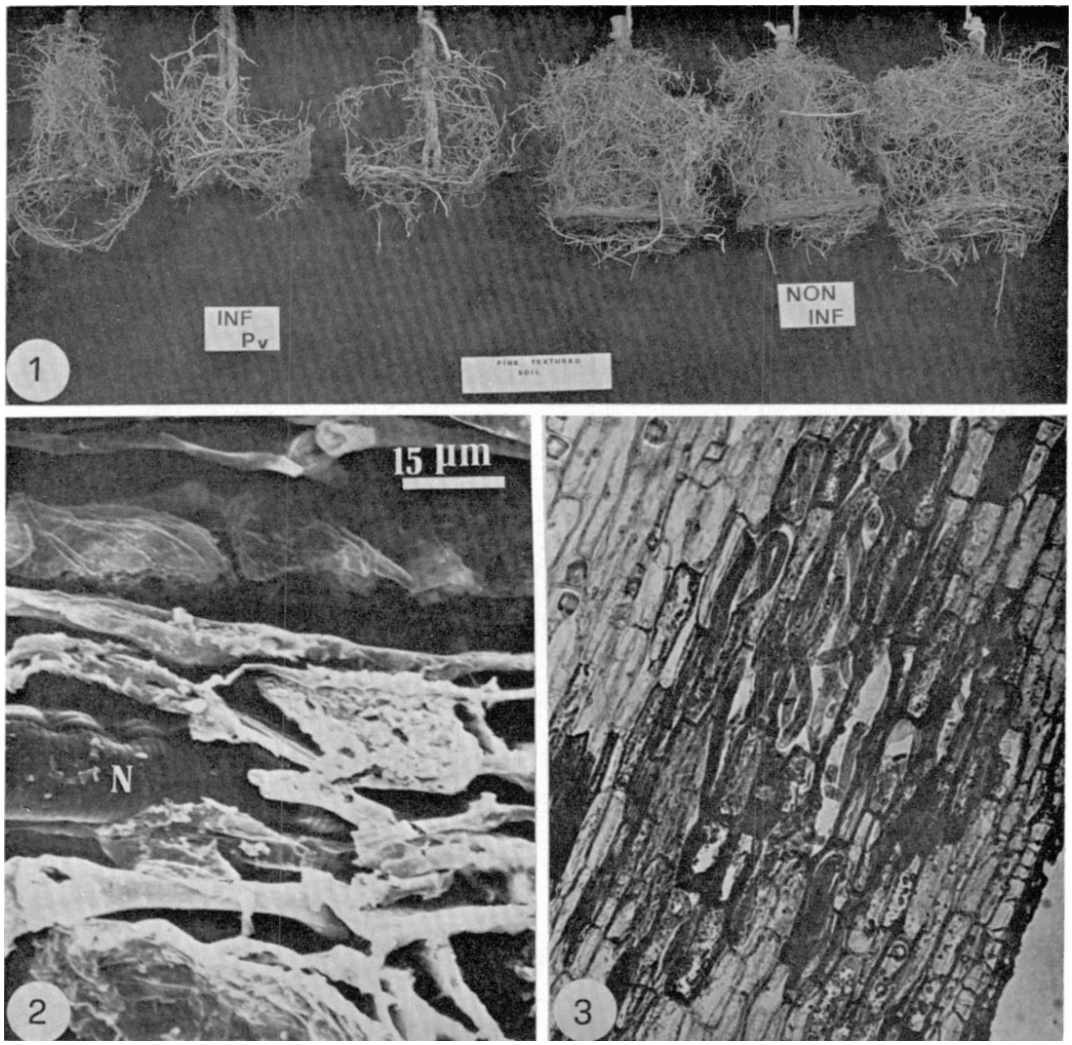

FIG. 1-3. *1)* Effects of *Pratylenchus vulnus* (PV) on roots of sour orange seedlings growing in fine textured soil (sandy clay loam). Three plants at left ("1NF") = infected; three plants at right ("NON INF") = controls. *2)* Longitudinal section of sour orange root showing coagulated cortical cells around a specimen of *P. vulnus* (N), (scanning electron microscope). *3)* Longitudinal section of sour orange root showing *P. vulnus* in a cavity in the cortical tissue.

severely suppressed (Fig. 1). Plant height, trunk diam., and dry root and top weights of inoculated plants were ($P \leq 0.05$) less than noninoculated controls (Table 2). In river sand, statistically significant growth retardation of inoculated seedlings occurred, in comparison with that of noninoculated seedlings (Table 2). The trunk diam. and dry root weights of inoculated plants were respectively 23 and 57% less than controls.

The numbers of *P. vulnus* extracted from plants grown in sandy clay loam at 6, 11, and 13 months were not significantly different among the three inoculum levels (Table 3). A higher nematode population density was observed 6 months after inoculation in the roots of seedlings grown in river sand than in sandy clay loam. This result might indicate that coarser soils favour early and rapid root invasion by *P. vulnus*. No substantial differences between nematode populations were detected in the two soil types at 11 and 13 months after inoculation (Table 3). These similar populations in the two soil types were the result of the damage being similar in both soils.

Cross sections of infected feeder root showed that *P. vulnus* usually invaded cortical tissue, an action which resulted in large cavities. Cells adjacent to cavities had

coagulated protoplasm which took the safranin stain (Fig. 2, 3). Occasional damage to the epidermal tissue was observed. No evidence of damage to stelar tissue was noted.

Pratylenchus vulnus caused stunting of sour orange seedlings similar to that caused by other *Pratylenchus* spp., especially *P. coffeae*. The behaviour of *P. vulnus* in relation to sour orange is similar to that reported for *P. coffeae* (11). Both nematodes are localized in cortical tissue, seldom invade vascular tissue, and suppress growth. Population densities of *P. coffeae* on rough lemon [*C. limon* (L.) Burm. f.] growing in fine and coarse textured soil (9) were analogous to the populations of *P. vulnus* on sour orange in these studies. *Pratylenchus vulnus* does not appear to be widespread in Italian citrus groves. However, investigations to detect its presence in nurseries and to adopt measures to avoid the spreading of this pest should be adopted by the citrus industry.

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