Effects of Pratylenchus vulnus on the Gnotobiotic Growth of Myrobalan Plum Seedlings

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ABSTRACT

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The growth and development of Myrobalan plum seedlings (cultivar 3J) maintained under gnotobiotic conditions for 10 weeks was inhibited by the root-lesion nematode, *Pratylenchus vulnus*. Root systems of nematode-infected plants were darker than those of noninfected controls and had many small lesions on feeder roots. Eggs, larvae, and adults were present in these lesions, and *P. vulnus* was feeding on healthy tissue at the lesion borders. Large secondary lesions did not develop during the 10 weeks of this experiment. The population density of *P. vulnus* increased more than 17-fold over the original inoculum level.

Additional key words: axenized nematodes.

Approximately 10% of the prune orchards in the Central Valley of California are infested with *Pratylenchus vulnus* Allen and Jensen (5). The principal rootstocks for prune, *Prunus cerasifera* var. Myrobalan 29C and *Prunus cerasifera* × *P. munsoniana* var. Marianna 2624, are hosts for *P. vulnus* (6). *Pratylenchus vulnus* has been associated with lesion formation on Myrobalan and Marianna selections in the field (1), and with disease in a variety of woody plants (8, 12). Reports of the pathogenicity of *P. vulnus* in the absence of all other organisms are lacking. This paper presents the results of such a study with Myrobalan plum.

MATERIALS AND METHODS

Isolation chamber.—A rigid-walled, positive-pressure isolation chamber (Germfree Laboratories, Inc., Miami, Florida) (Fig. 1), 75 cm high and enclosing a $50 \times 120\ cm^2$ area, was used in these studies. Air entering and leaving the chamber was filtered (4). Heat-resistant items needed for this experiment were autoclaved in a vented, stainless steel cylinder (4). After autoclaving, the cylinder was attached to the chamber air-lock portal with a vinyl connecting sleeve. This portal was used to introduce items into the chamber. A frame consisting of eight 1.83 m Sylvania Gro-Lux fluorescent lights was suspended over the chamber. Light intensity varied from 7500 lux at the chamber top to 5400 lux at the bottom. The plants were exposed to a 16-hour day. Temperatures ranged from 28 C (in light) to 20 C (in dark).

The chamber walls, air-lock, and connecting sleeves were disinfested by first washing the exposed surfaces with 2% peracetic acid + 0.01% di-octyl sodium sulfosuccinate solution and then spraying with 5% peracetic acid-surfactant solution. The unit was then left undisturbed for 48 hours with the air blower (4) operating to dissipate the peracetic acid before use. Media used for detection of contamination in the chamber were Czapek-Dox, brain-heart infusion, and thioglycollate broths. Random samples were taken every 2 weeks from the chamber interior, potting medium, and plant materials.

Water for the plants was obtained from a siphon apparatus located outside the chamber. This apparatus consisted of two 18-liter glass bottles containing sterile distilled water. Another 18-liter bottle containing a water-soluble fertilizer [Hyponex® (The Hyponex Co., Inc., Copley, Ohio): 5.14 g/3,785 ml distilled water] was connected to a common tube with the water lines. This common tube was connected to a Lucite® (E. I. dePont de Nemours and Co., Wilmington, Delaware) tube which extended through the chamber wall.

Axenic procedures.—A modification of several methods (2, 3, 10, 11) of after-ripening of seeds was used to attain apparent microorganism-free Myrobalan 3J seedlings. Intact seeds were immersed in 95% ethanol for 50 seconds, agitated for 45 minutes in a solution containing 100 ml of commercial chlorine bleach and 10 ml of liquid detergent (Ivory Liquid®, Proctor and Gamble Co., Cincinnati, Ohio) diluted to one liter, rinsed three times in sterile distilled water, and placed in a sterile, cotton-plugged 250-ml flask containing 5 ml of sterile distilled water. After 5 days the suture between each half of the endocarp became soft, and the seed could be removed easily without damaging the integument. The above procedure was repeated with the exposed seed, except that agitation in the chlorine solution was reduced to 15 minutes. After 5 days, the integument, endosperm, and nucellus were carefully removed. The excised embryos were surface-disinfested by soaking in the chlorine solution for 3 minutes, rinsed thoroughly with sterile distilled water, and placed on an agar medium

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consisting of 10 g agar, 5 g glucose, 1 mg vitamin B_1, and 1.5 g of a dry salts mixture (3 g potassium chloride, 1.5 g ferric chloride, 5 g potassium nitrate, and 2.5 g each of calcium sulfate, magnesium sulfate, calcium phosphate) per liter of distilled water.

After growth on this medium for 4 or 5 days in darkness, the embryos were placed in a lighted transfer chamber. Radicles and plumules reached a length of approximately 5 cm in 2 weeks. Seedlings apparently free of microorganisms were placed in a stoppered flask containing 250 ml of sterile distilled water and introduced into the chamber. To check for contamination the seedlings were plated on 20 ml of the agar medium described above.

After 15 days, eight apparently axenic seedlings were planted singly to 16-cm diameter clay pots containing 1.25 liters of a sand-vermiculite (5:1, v/v) potting medium. Iron, magnesium, and zinc chelates were applied 5 days after the seedlings were transplanted into the pots. Two weeks after transplanting, anomalous growth of the seedlings was evident (e.g., crinkled leaves, slight petiole twist, and no elongation at the growing points). Three foliar applications of 35 μg/ml gibberellic acid applied at 5-day intervals alleviated this condition (2). Thirty days after transplanting, bacteria were detected in one of the eight pots. It was removed from the chamber, and the site it had occupied was treated with 100 ml of a solution containing 2,000 μg penicillin and 6,000 μg dihydrostreptomycin sulfate per ml. No other microorganisms were detected in subsequent test samples.

After 6 weeks, the plum seedlings were grouped in three similar pairs based on size. The seventh plant was removed from the chamber, and one plant in each of the pairs was inoculated with a 50-ml aqueous suspension of 1,600 axenized *P. vulnus* (9) poured into holes opened into the potting medium around the root zone. The three controls received 50 ml of sterile, distilled water. Ten weeks after inoculation the plants were removed from the chamber, and the heights and weights of the tops of each plant measured. The root systems were separated from the potting medium, washed free of soil, blotted dry, and weighed. Nematodes were extracted from roots and the potting medium by incubation under intermittent mist (6). Small portions of roots were selected at random, stained in acid fuchsin-lactophenol, cleared in lactophenol alone (7), and examined microscopically.

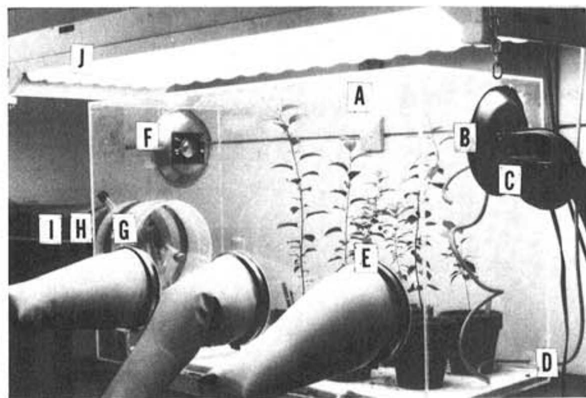

Fig. 1. Apparatus used for gnotobiotic culture of Myrobalan plum seedlings infected with *Pratylenchus vulnus*. Rigid-walled isolation chamber (A) showing the positive-pressure air filtration system (B, C, F), water-fertilizer delivery tube (D), gloves (E), air-lock portal (G), connecting sleeve (H), stainless steel cylinder (I), and lighting system (J).

TABLE 1. Size of gnotobiotically grown Myrobalan 3J seedlings and numbers of nematodes 10 weeks after inoculation with 0 or 1,600 axenized *Pratylenchus vulnus*

No. of *P. vulnus*				
Added (per pot)	Recovered (per g of root)[a]	Plant height (cm)[a]	Top weight (g)[a]	Root weight (g)[a]
0	0	64	8.7	6.0
1,600	27,715	33	3.2	1.4
LSD ($P = 0.05$)		21	7.4	5.5
LSD ($P = 0.10$)		15	5.3	3.5

[a]Average of three replicates.

RESULTS AND DISCUSSION

Plants parasitized by *P. vulnus* under gnotobiotic conditions were smaller than nematode-free controls (Table 1). Their root systems were darker in color, had fewer feeder roots, and had small lesions on many of the primary feeder roots. Ten to 20 *P. vulnus* usually were associated with each lesion. When fewer nematodes were present, only a few discolored cells were visible. Many dead feeder roots were recovered from the potting medium of plants infected by *P. vulnus*. Adults, larvae, and eggs of *P. vulnus* were present in the cortex of primary roots. The nematodes were aligned parallel with the long axis of the root and were feeding in healthy tissue at the borders of the lesions. The population density of *P. vulnus* increased more than 17-fold over the original inoculum level in 10 weeks (Table 1). Apparently the feeding and reproduction of *P. vulnus* in the cortex of primary roots caused tissue disintegration, which led to lesion formation, death of feeder roots, and reduction in plant weight. The lesions which were associated with the cortex of the primary feeder roots were much smaller than those caused by *P. vulnus* on plum in the field (1). We have observed that large root lesions associated with *P. vulnus* are formed after >6 months of exposure to the nematode on secondary roots. It is possible that this time is required for either root differentiation from primary to secondary roots or for accumulation of sufficient phenolic substrates. The 10-week duration of this gnotobiotic study was insufficient for large lesion production. In this test, and in the field, we have observed stunting of seedlings before large lesions were formed. This stunting probably resulted from destruction of feeder roots and consequent impairment of root function.

It is difficult, for many reasons, to relate nematode population dynamics in pot experiments to those in the

field. However, the inoculum level used in this experiment (1.3 *P. vulnus* per cm^3 soil) was no higher than is commonly found around host roots in the field.

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