Effects of *Pratylenchus vulnus* and *Xiphinema index* Singly and Combined on Vine Growth of *Vitis vinifera*[1]

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Abstract: Inoculation of 'Thompson Seedless' grapevines with 500 *Xiphinema index* or 1,000 *Pratylenchus vulnus* alone or in combination suppressed vine shoot and root growth under greenhouse conditions. *Pratylenchus vulnus* caused greater stunting of roots than *X. index*. Each nematode species inhibited top growth about equally. Concomitant inoculations caused greater stunting of tops and roots than did inoculations of either nematode species alone. Differences in growth between inoculated and control plants increased with exposure time. *Pratylenchus vulnus* competed with and gradually superseded in numbers an established population of *X. index*. Both species reproduced on 'Thompson Seedless' roots, but *P. vulnus* increased to a much higher level than did *X. index*. The increase of *P. vulnus*, together with extensive damage, proves its pathogenicity to grapevines. *Key Words:* nematode interaction.

Various root-lesion nematodes of the genus *Pratylenchus* have been reported in roots of grapevines in California (1, 17, 18, 26). Among these is *Pratylenchus vulnus* Allen and Jensen, which has been found to cause severe injury to peach, citrus, narcissus, walnut, plum, boxwood, rose, avocado, and ponderosa pine (2, 4, 5, 7, 9, 14, 15, 19, 22, 23, 25). *Xiphinema index*, a known grape pathogen (3, 8, 16, 18, 24), has been found in mixed populations with *P. vulnus*. Information on the effect of the lesion-nematode on grapevines is limited (18, 20) and, regarding mixed populations

Received for publication 23 March 1976.

[1] Portion of the senior author's Ph.D. dissertation.

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with *X. index,* unavailable. The purpose of this study was to determine the pathogenicity of *P. vulnus* on the grape variety 'Thompson Seedless' and to study its effects in concomitant inoculations with *X. index* under greenhouse conditions.

MATERIALS AND METHODS

Two-bud cuttings of 'Thompson Seedless' grapevine, *Vitis vinifera,* were obtained from virus-free canes. Cuttings were rooted in sterile sand and transplanted after 1 month to 20-cm clay pots of autoclaved sandy loam soil.

Fungi associated with the grape cuttings and potted soil were isolated and identified by being placed on three differential media: potato-dextrose agar (PDA), cornmeal agar (CMA), and water agar (WA). These preparations were incubated at 24 C for 6-10 days. Fungi were isolated, established in pure culture, and identified. Similar isolations from potted soil were made at 135, 255, and 362 days after inoculation from all treatments.

The original source of *P. vulnus* was a walnut host, *Juglans regia,* in Escalon, San Joaquin County, California. The population was increased on carrot slices (13) and surface sterilized (10) prior to the inoculation of grape cuttings. The solution for sterilization was modified by adding 100,000 units of Penicillin G in 70 ml of sterile water.

Virus-free *X. index* was used for inoculum. The population was originally isolated from grapevine in Christian Brothers vineyards, Napa County, California and raised on fig, *Ficus carica,* for 3 years under greenhouse conditions. Only nematodes recovered on 250- and 148-μm sieves were used in the experiment. A higher concentration of Aretan® (200 μg/ml) and an antibiotic (200,000 units of Penicillin G in 70 ml of sterile water) was tried (unsuccessfully) to axenize *X. index.* Accordingly, the wash water remaining after *X. index* was removed by combined screening, and microscopical examination was included as an inoculation treatment (CMAX).

Three separate experiments were inoculated in April 1974, and each was harvested on a different date (135, 255, and 362 days following inoculation). Each experiment included 7 treatments and 9 replications in a randomized block design. The treatments were: *(i)* Check, *(ii)* Supernatant check on microorganisms associated with *X. index* (CMAX), *(iii)* 500 *X. index, (iv)* 1,000 *P. vulnus, (v)* 500 *X. index* and 1,000 *P. vulnus* added at the same time, *(vi)* 500 *X. index* added first and 1,000 *P. vulnus* 1 month later; *(vii)* 1,000 *P. vulnus* added first and 500 *X. index* 1 month later. Initial inoculations were made 10 days after transplanting.

Twenty-cm clay pots were embedded in sawdust and irrigated at 1-3 day intervals with distilled water as needed. In addition, 250 ml of half-strength Hoagland's nutrient solution were added weekly. Soil temperature was maintained between 20-30 C. The 362-day experiment had a 5-week dormant period from late December to early February. During that time, heaters were turned off and soil temperature dropped to 5-16 C. The plants, which went into dormancy, were cut back. Pests and diseases, especially white flies, mites, leaf rollers, and powdery mildew, were controlled in all experiments.

At the end of the experiment, surface-dried top and root weights were recorded. Surface areas of the leaves were determined indirectly by printing their outlines on Xerox paper and weighing the cut shapes. The leaf areas were determined by multiplying the weights by the area of a unit weight of the paper.

Nematode densities in soil and roots were measured at the end of each experiment. The root system was washed free of soil particles with a known volume of water and stirred for 1 min. A volume of 300 cm^3 of the slurry was used as a sample, and nematodes were extracted by sugar-flotation (6). Total numbers of nematodes in the soil were then calculated.

The whole root system was finely chopped, added to a pan of water, and stirred to obtain an homogeneous sample. Ten grams of chopped root material were placed under intermittent mist (10) for 15 days. Nematode counts were made every 3 days, and total numbers of nematodes in roots were calculated.

RESULTS AND DISCUSSION

The numerous fungi isolated from the grape cuttings prior to rooting and from the soil were ubiquitous organisms found in or on a wide array of substrates. There were no grape pathogens among them.

Root weights, top weights, and surface area of the leaves of checks and CMAX were greater than those of inoculated treatments at 135, 255, and 362 days after inoculation ($P = 0.05$). There were no differences between checks and CMAX (Tables 1, 2). Roots from plants in the check and CMAX showed abundant formation of secondary roots with yellow and brown cortex; however, some roots appeared dead because of normal senescence. Plants in pots exposed to *X. index* showed swollen root tips, which are typical of nematode feeding (3, 16). An abundance of secondary roots was generally present. Root systems of plants from treatments with exposure to *P. vulnus* showed a lack of secondary roots and a darkened cortex (9, 12, 14, 20). Symptoms were more severe on plants inoculated with both nematodes, and top and root weights were lowest in these treatments. At 362 days, plants treated with *P. vulnus* alone and in combination with *X. index* were more stunted than plants treated with *X. index* alone (Fig. 1, Table 1). Plants with double inoculations also showed a cumulative suppression in shoot growth [top weight (Table 1) and leaf surface area (Table 2)] in comparison to plants inoculated with a single nematode species. This stunting was most evident at 362 days and may have been due to both nematode species.

Four instances of contamination were recorded during the examination of plant roots from the three experiments. These were probably due to nematode eggs being filtered through the 44-μm sieve in CMAX, and the occurrence of cross contamination by irrigation because pots were close together. These instances were treated as missing plots in the statistical analysis.

The low number of nematodes found in all inoculated treatments at the end of the third experiment (Fig. 2) supports an earlier hypothesis (21) that prolonged nematode feeding activity reduces the supply of food and, consequently, the nematode population. This decline of the nematode population also might have resulted from a sensitivity to temperature

TABLE 1. Influence of *Pratylenchus vulnus* and *Xiphinema index* (singly and combined) on growth of 'Thompson Seedless' grapevine.

Treatment	Fresh weight (gm)/harvest (days after inoculation)[v]		
	135 days	255 days	362 days
Tops			
Check	67 a	115 a	149 a
CMAX[w]	66 a	121 a	140 a
X. index	30 b	67 b	86 b
P. vulnus	30 b	57 b	66 bc
Xi + Pv[x]	23 b	51 bc	46 cd
Xi then Pv[y]	27 b	50 bc	49 cd
Pv then Xi[z]	28 b	35 c	36 d
Roots			
Check	176 a	291 a	309 ab
CMAX[w]	174 a	284 a	336 a
X. index	128 bc	185 b	265 b
P. vulnus	134 b	143 bc	190 c
Xi + Pv[x]	110 cd	144 bc	153 c
Xi then Pv[y]	117 bcd	152 bc	172 c
Pv then Xi[z]	102 d	125 c	149 c

[v]Means of nine replicates. Values in each column followed by the same letter do not differ ($P = 0.05$, Duncan's Multiple Range Test).
[w]Check on the micro-organisms associated with *X. index*.
[x]*Xiphinema index* (500) and *P. vulnus* (1,000) added at the same time.
[y]*Pratylenchus vulnus* added 1 month after *X. index*.
[z]*X. index* added 1 month after *P. vulnus*.

TABLE 2. Leaf growth of 'Thompson Seedless' grapevines as influenced by *Xiphinema index* and *Pratylenchus vulnus*.

Treatment	Leaf growth in cm^2/harvest[v]		
	135 days	255 days	362 days
Check	1,984 a	3,124 a	1,383 a
CMAX[w]	1,952 a	3,082 a	1,208 a
X. index	1,194 b	1,693 b	900 b
P. vulnus	1,098 b	1,472 bc	729 bc
Xi + Pv[x]	1,006 b	1,290 bc	501 d
Xi then Pv[y]	992 b	1,340 bc	540 cd
Pv then Xi[z]	875 b	1,086 c	426 d

[v]Means of nine replicates. Values in each column followed by the same letter do not differ (Duncan's Multiple Range Test, $P = 0.05$).
[w]Check on the microorganisms associated with *X. index*.
[x]*X. index* and *P. vulnus* added at the same time.
[y]*P. vulnus* added 1 month after *X. Index*.
[z]*X. index* added 1 month after *P. vulnus*.

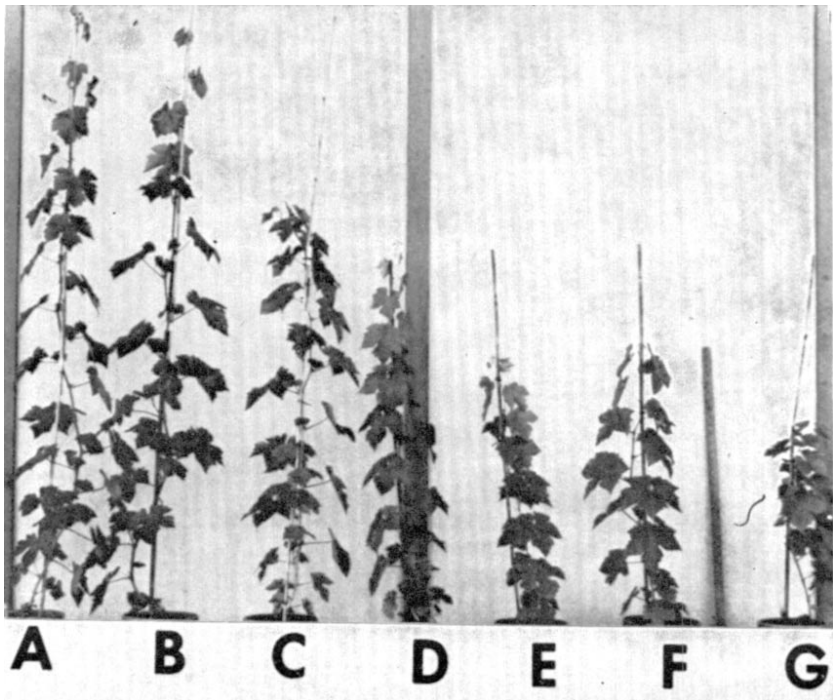

FIG. 1 (A-G). Grape plants variety 'Thompson Seedless' 362 days after inoculation. *A)* Check. *B)* CMAX. *C)* 500 *Xiphinema index*. *D)* 1,000 *Pratylenchus vulnus*. *E)* 1,000 *P. vulnus* and 500 *X. index* added at the same time. *F)* 500 *X. index* added first and 1,000 *P. vulnus* 1 month after. *G)* 1,000 *P. vulnus* added first and 500 *X. index* 1 month after.

variations during the 5-week period, from late December to early February 1975, in which greenhouse heaters were turned off and the temperatures dropped to 5-16 C. During this period, plants remained dormant.

The population of *P. vulnus* increased 162-fold after 135 days when it was added singly to the 'Thompson Seedless' grapevines (Fig. 2-B). Lownsbery and Serr (11), however, found that the same grape variety was a poor host for *P. vulnus*. Conditions under which their host range study was carried out, such as smaller pots (15-cm) placed in a lathhouse, might have accounted for their findings.

The presence of *P. vulnus* inhibited ($P = 0.01$) the increase of *X. index* at the end of the growing season (255 days) (Fig. 2-A). This suppression occurred whether *P. vulnus* inoculation was before or after *X. index* inoculation. Extensive damage, resulting from active feeding by *P. vulnus* in cortex tissues, destroyed secondary roots. Destruction of roots reduced the number of feeding sites for *X. index*, although no

TABLE 3. Final number of nematodes per pot following inoculation with either 500 *Xiphinema index* or 1,000 *Pratylenchus vulnus* or both.

Treatments		Numbers (in 1,000's) of *X. index* and *P. vulnus*/pot at different harvest dates[v]		
		135 days	255 days	362 days
Check		0	0	0
CMAX[w]		0	0	0
X. index		30	34	2
P. vulnus		162	104	44
Xi then Pv[x]	Xi	17	8	6
	Pv	153	172	65
Xi then Pv[y]	Xi	11	14	10
	Pv	93	103	85
Pv then Xi[z]	Xi	21	12	6
	Pv	84	132	33

[v]Means of nine replicates.
[w]Check and check on the associated microorganisms associated with *X. index* (CMAX) had no nematodes except two contaminated plants.
[x]*X. index* and *P. vulnus* added at the same time.
[y]*P. vulnus* added 1 month after *X. index*.
[z]*X. index* added 1 month after *P. vulnus*.

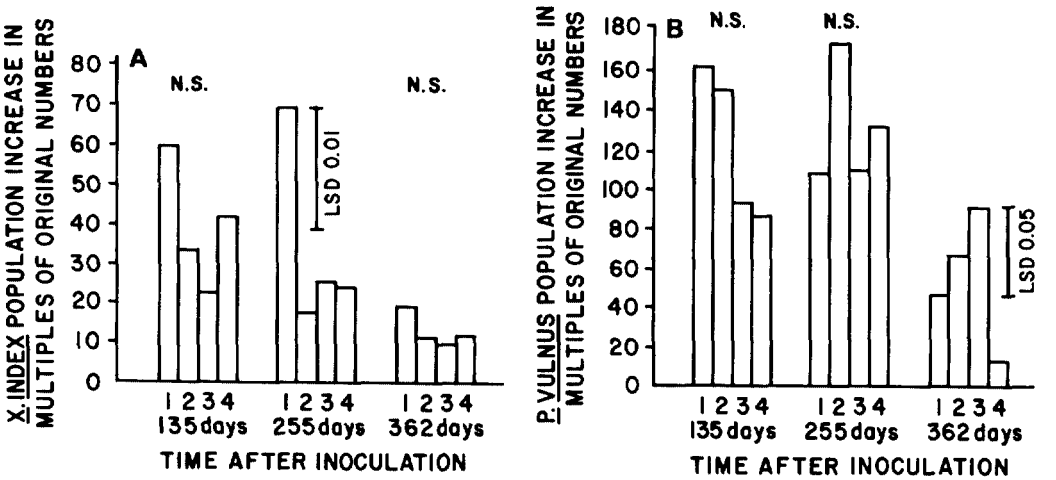

FIG. 2 (A-B). Increase of *(A) X. index* and *(B) P. vulnus* populations when each species was added alone compared to increase when both species were added in joint inoculations on grape var. 'Thompson Seedless' at three harvest dates. Initial inoculation levels were 500 *X. index* and 1,000 *P. vulnus*. 1 = *P. vulnus* added alone; 2 = *X. index* and *P. vulnus* added at the same time; 3 = *P. vulnus* added 1 month after *X. index;* 4 = *X. index* added 1 month after *P. vulnus*.

direct competition for feeding sites was observed. Root deterioration possibly increased with the presence of saprophytic fungi and bacteria. *Pratylenchus vulnus* also showed a higher rate of reproduction than *X. index* (Fig. 2) which probably would allow this species to utilize available food supply at a greater rate. The limited feeding sites did not permit *X. index* to reach as high a density as the nematode did when *P. vulnus* was absent. Formation of polyphenolic compounds stimulated by *P. vulnus* also might have inhibited or retarded increase of *X. index*. On the other hand, populations of *P. vulnus* were not affected by *X. index* at any sampling date. When present alone, *P. vulnus* reached the "ceiling level" at 135 days or before, and when present in combination, at 255 days (Fig. 2-B). Nematode populations fluctuated widely throughout the three experiments.

Pratylenchus vulnus had an effect equivalent to that of *X. index* in the suppression of shoot growth, and a greater effect on root growth when single inoculations by each nematode were compared. In combination, their effect was more damaging. *Pratylenchus vulnus* can satisfactorily compete wth an established population of *X. index* and largely supersede it in numbers. When the effects from the two nematode species are compared alone and in combination, *P. vulnus* seems to have accounted for the greater growth retardation. Buildup of this nematode and the extensive damage to the plants following inoculation proves its pathogenicity to grapevine and supports the many early reports, based on field observation of damage by this organism (1, 12, 17, 18, 20).

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