Effects of *Paratrichodorus minor* (Colbran) Siddiqi on Thompson seedless grapevine

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SUMMARY

Root and shoot weight of seedlings of *Vitis vinifera* cv. Thompson Seedless grapevine grown twenty months in the greenhouse in soil infested with 100, 1 000, and 10 000 *Paratrichodorus minor* (Colbran) Siddiqi, were significanly less than weights of uninfected control seedlings. *P. minor* reproduced well on Thompson Seedless grape. Chimaera symptoms whose etiology is uncertain also were found, mostly at the higher nematode inoculum levels.

RÉSUMÉ

Effets de Paratrichodorus minor *(Colbran) Siddiqi sur la vigne, cv. Thompson seedless*

Chez de jeunes plants de vigne *(Vitis vinifera)* cv. Thompson seedless cultivés pendant vingt mois en pépinière sur du sol infesté par 100, 1 000 et 10 000 *Paratrichodorus minor* (Colbran) Siddiqi, les poids des racines et des parties aériennes étaient significativement inférieurs à ceux des plants témoins. *P. minor* se reproduit bien sur la vigne, cv Thompson seedless. Des symptômes de chimères, d'étiologie incertaine, ont été observés, surtout aux plus hauts niveaux d'inoculation.

The stubby-root nematode *Paratrichodorus minor* (Colbran) Siddiqi is an ectoparasitic nematode with wide geographical and host range which causes damage to perennial crops by attacking the root tips of host plants (Khera & Zuckerman, 1963 ; Rhode & Jenkins, 1957 ; Ruehle, 1969 ; Standifer & Perry, 1960 ; Zuckerman, 1963). *P. minor* has been found in soil around the roots of grapevine throughout California (Raski, 1955 ; Raski & Radewald, 1958), but its pathogenicity to grapevine has not been studied. Because of the importance of grapes in California agriculture and the apparently wide-spread distribution of the stubby root nematode, experimental tests were begun to determine what influence this nematode might have on the growth of grapevines.

Materials and methods

A population of *P. minor*, originally isolated from grapevine in Temecula, Riverside County, California, was increased on that host in the greenhouse and then maintained on Thompson Seedless grapevine.

Nematode inoculum was extracted from soil by Cobb's (1918) method and placed on a Baermann funnel under a heated, intermittent mist for 48 hours (Lownsbery & Serr, 1963). Inoculations were made by pipetting aliquots of aqueous suspensions with the desired number of *P. minor* into four holes 4-6 cm deep and 5 cm from the crown of the plants.

Dormant two-bud cuttings of *Vitis vinifera* cv. Thompson Seedless were obtained from virus-

free canes ; these were placed in cold storage until mid-February. Cuttings were rooted in flats of autoclaved sand and placed in a growth chamber for four weeks. Rooted cuttings were transplanted singly into an autoclaved mixture of one-third soil and two-thirds sand in 20 cm diam. clay pots. These plants were kept in the greenhouse to establish and develop good root systems before inoculation. Established eight-week old plants were given several inoculation treatments, and the replicates were arranged in nine randomized blocks in a greenhouse. Treatments were : 1) Uninoculated control (water only added) ; 2) A control to measure the effect of micro-organisms other than nematodes in the suspension : this was obtained by screening a a portion of the nematode suspension (the 10 000 nematode level) five times through a sieve with 0.025 mm openings and hand-picking out any nematodes remaining in the suspension ; 3), 4), and 5) *P. minor* at inoculum levels of 100, 1 000, and 10 000 nematodes per pot ; and 6) 10 000 nematodes given a surface sterilization treatment for 6 h in a solution containing 130 ppm Aretan (Plant Protection Limited, London, England) then rinsed once in sterile water (Goodman & Chen, 1967).

Pots were placed on benches in the greenhouse during the growing season and artificial light was supplied 12 h a day to supplement the short day period of the fall months (October-December). This was done to avoid premature dormancy. The greenhouse temperature was maintained between 20-25°. Plants were moved from the greenhouse benches to sawdust beds in the lathhouse for two months during the winter. Plants were irrigated with distilled water as needed, and with half-strength Hoaglands's nutrient solution added once a week.

When plants commenced growth the second growing season they were pruned to two buds. Shoot weights and heights were measured 65, 170, 335 and 440 days after this pruning. After twenty months, roots were carefully washed free of soil, blotted dry, weighed and photographed. The entire soil mass from each pot was placed in a pan where the soil was grossly removed from the roots and carefully mixed for uniformity. Then, by using a minimum amount of water, the roots were washed free of any remaining soil in a second pan. One-half of the soil from the first pan was then mixed carefully into the contents of the second pan. The entire contents of pan two were then screened according to Cobb's method and the sievings placed on a Baermann funnel in a mist chamber for 48 hours. The number of nematodes was used as a relative measure of the number of nematodes per replicate.

Data were subjected to analysis of variance, and differences between means were distinguished using L.S.D. test and Duncan's multiple range test.

Results and discussion

The heights of shoots and fresh weights of shoots and roots were significantly less than in the uninoculated controls (Tab. 1, 2 & 3 ; Figs 1 & 2). Root and top weights and shoot heights of the uninoculated controls were greater than those of inoculated treatments ($P = 0.01$). Root and shoot weights and top heights of the control for associated microorganisms did not differ significantly from uninoculated controls. Roots from uninoculated control plants and associated microorganism control plants showed abundant formation of secondary roots with yellow and brown cortex. The surface-sterilization with $HgCl_2$ did not alter the pathogenicity of *P. minor*. Root systems of inoculated plants were smaller and showed a lack of normal secondary feeder roots and a darkened cortex.

Fresh weights and heights of shoots showed a different response to different inoculum levels in each of the four cuttings (Tab. 1 & 2). The first cutting, nine months after inoculation and 65 days after the dormant season, showed statistically significant differences between the uniniculated control and the three different inoculum levels, but there were no significant differences between the 1 000 and 10 000 nematode inoculum levels. In the second cutting, twelve months after inoculation, there was greater reduction at the 1 000 nematode inoculum level than at the 100 and 10 000 levels. This may have resulted because 100 nematodes had not increased to a damaging level. The 10 000 nematodes may have declined because of insufficient root substrate for this level (Seinhorst, 1961). The heights and weights of the

Table 1

Fresh weight of shoot cuttings of Thompson Seedless grapes at intervals after inoculation with different numbers of *Paratrichodorus minor*

Inoculum	*Weights (g) of shoot cuttings Number of days after 2nd season pruning*				
	65	*170*	*335*	*440*	*Total* *
Uninoculated Control	42	59	46	53	200 a
Associated microorganism Control	33	55	53	47	188 a
100 *P. minor*	20	39	37	32	128 b
1 000 *P. minor*	14	29	30	29	102 c
10 000 *P. minor*	14	36	27	28	105 c
10 000 *P. minor* Surface sterilized	14	36	27	30	107 c

Cutting	*L.S.D. 5%*	*L.S.D. 1%*
1st	4.32	5.79
2nd	5.52	7.39
3rd	4.25	5.70
4th	7.95	10.63

* Mean of 9 replicates.
Values in each column not followed by the same letter differ significantly at the 5% level (Duncan's Range Test).

Table 2

Linear growth of shoots of Thompson Seedless grapes at intervals after inoculation with different numbers of *Paratrichodorus minor*

Inoculum	*Linear growth of cuttings in cm Number of days after 2nd season pruning*				
	65	*170*	*335*	*440*	*Total* *
Uninoculated Control	70	171	146	100	487 a
Associated microorganism Control	60	145	151	98	454 a
100 *P. minor*	38	118	100	68	324 b
1 000 *P. minor*	31	97	78	61	267 b
10 000 *P. minor*	29	109	75	56	269 b
10 000 *P. minor* Surface sterilized	30	101	75	63	269 b

Cutting	*L.S.D. 5%*	*L.S.D. 1%*
1st	6.84	9.14
2nd	17.40	23.28
3rd	10.50	14.03
4th	14.70	19.64

* Mean of 9 replicates.
Values in each column not followed by the same letter differ significantly at the 5% level (Duncan's Range Test).

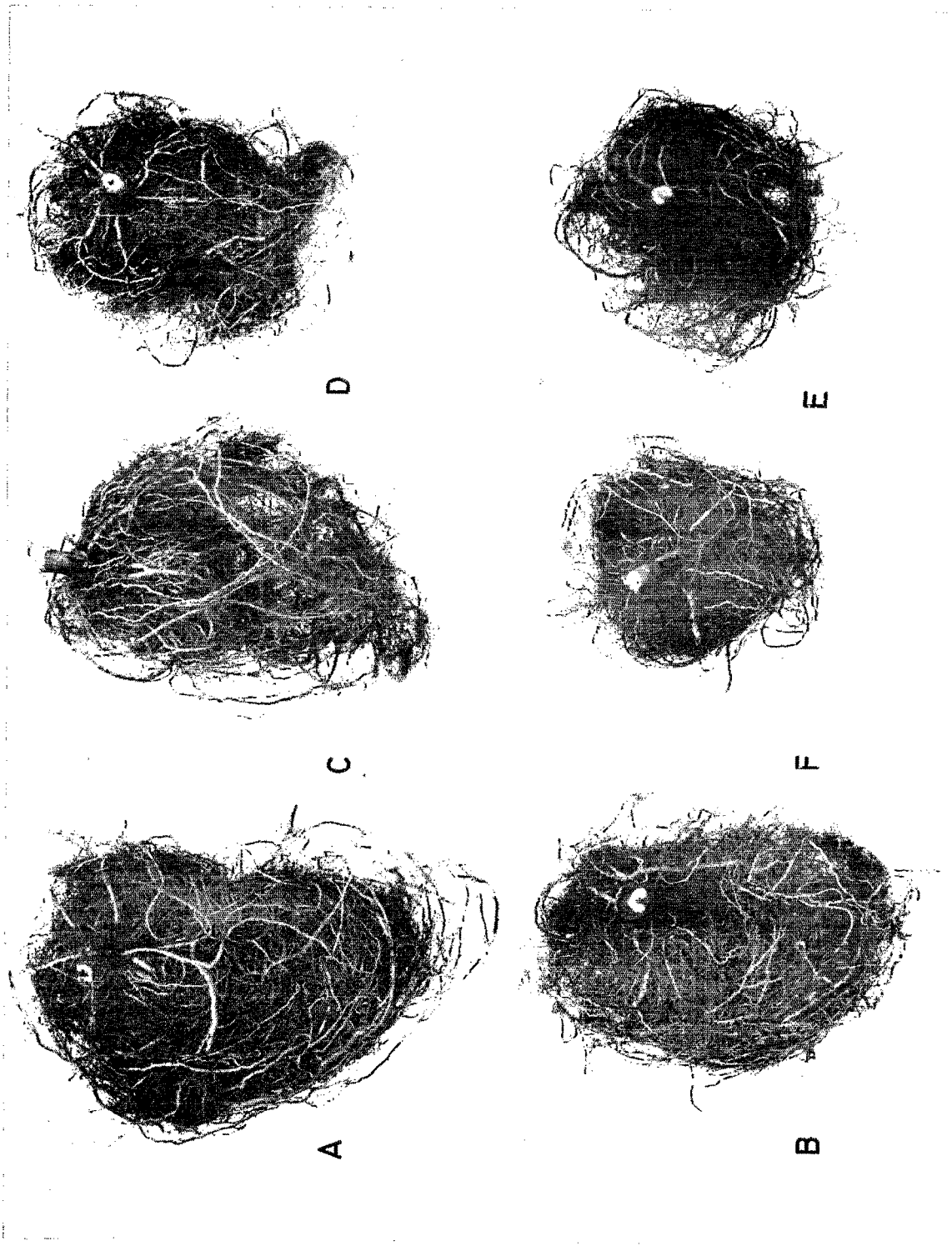

Fig. 1. (A-F) Roots of Thompson Seedless grape 600 days after inoculation with *Paratrichodorus minor*: a) Uninoculated control; b) Associated microorganism control; C) 100; D) 1 000; E) 10 000; F) 10 000 surface sterilized with Hg Cl_2.

third and fourth cuttings ranked similarly to the second cutting.

Table 3

Numbers of *Paratrichodorus minor* per pot 20 months after inoculation and root fresh weight of Thompson Seedless grapes

Inoculum	*Number of* P. minor/*pot* *	*Root Fresh Weight* *
Uninoculated Control	—	122 a
Associated micro-organisms Control	—	118 a
100 *P. minor*	1 999	81 b
1 000 *P. minor*	4 391	81 b
10 000 *P. minor*	7 373	69 c
10 000 *P. minor* Surface sterilized	8 362	67 c

* Mean of 9 replicates.

Values in each column not followed by the same letter differ significantly at the 5% level (Duncan's Range Test). Log_{10} transformation of data corrected heterogeneity of data before analysis of variance.

P. minor reproduced well on Thompson Seedless grapevine growing in a mixture of sand and soil, (2 : 1, respectively, Tab. 3). This is at variance with the report of Raski and Radewald (1958). They found *Trichodorus christiei* (= *P. minor)* failed to reproduce on the same host grown in pure soil. The low number of nematodes found in the higher inoculum level at the end of the experiment indicates that prolonged nematode feeding activity reduces the supply of food and, consequently, the nematode population. This decline of the nematode population might have resulted from sensitivity to temperature variation during the eight weeks period during which the plants were moved to the lathhouse and temperature dropped to 5- 16° (Bird & Mai, 1967). During this period, plants remained dormant. Chimaera symptoms (several kinds of partial chlorophyll defects) also were found, mostly at the higher nematode inoculum levels (Fig. 3). The same chimaera symptoms were observed when culturing Thompson Seedless grapevine in the greenhouse without nematode in sand using distilled water for irrigation. The chimaera symptoms disappeared later after fertilization with half strength Hoagland's nutrient solution. This suggest that chimaera appearance may be related to nutritional stress resulting from nematode infection as reported on tomato by Maung and Jenkins (1959).

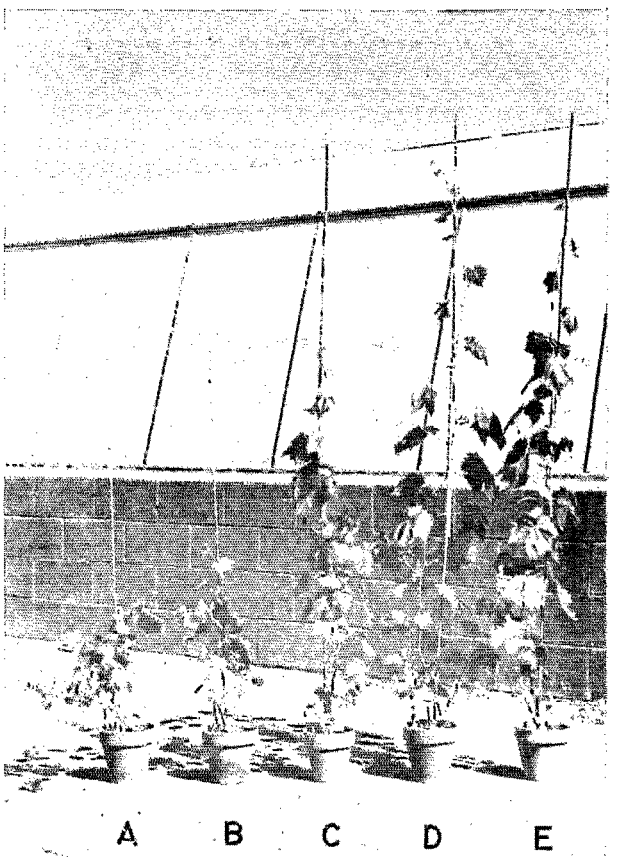

Fig. 2. Shoot growth of Thompson Seedless grape immediately before the second cutting 300 days after inoculation with *Paratrichodorus minor* : A) 10 000 ; B) 1 000 ; C) 100 ; D) Associated microorganism control ; E) Uninoculated control.

The evidence shows Thompson Seedless grapevine is a host for *P. minor*. The evidence also suggests that the stubby root nematode *P. minor* is pathogenic to *Vitis vinifera* cv. Thompson Seedless grapevine because root and shoot weights were reduced significantly at all levels of inoculation during a period of twenty months. It is particularly notable that significant damage was caused by inoculation by only 100 *P. minor*.

Fig. 3. Chimaera symptom on Thompson Seedless grapevine infected with *Paratrichodorus minor* for 300 days.

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