Journal of Nematology 16(2):140–145. 1984.

Pathogenicity of *Criconemella curvata* to Alfalfa

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Abstract: Large populations of *Criconemella curvata* and extended experimental periods were required to adversely affect yield of 'Moapa 69' alfalfa. *C. curvata* ectoparasitic feeding caused a reduction in feeder root numbers and tap root size and small lesions on tap and secondary roots. Greater reproduction occurred at 27 C than at 22 C, but the effect of the nematode on alfalfa growth was the same at both temperatures.
Key words: ring nematodes, lucerne, temperature, feeding.

Alfalfa (*Medicago sativa* L.) stand decline is the most serious production problem facing alfalfa producers in California. Decline has a complex etiology which may involve many different pests including several species of nematodes. Association of *Criconemella curvata* (Raski) Luc & Raski with alfalfa in the United States has not been reported previously. We recovered *C. curvata* from an alfalfa field near Manteca, California, which was also infested with *Meloidogyne hapla* Chitwood, *M. javanica* (Treub) Chitwood, and *M. incognita* (Kofoid & White) Chitwood. Some of the cultivars in the field were resistant to one or more of the root-knot nematode species; when these cultivars began to die out, the role of *C. curvata* in the stand decline was questioned.

C. curvata is widely distributed and associated with forage crops in eastern Can-

Received for publication 3 June 1983.
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ada (17) and red clover (*Trifolium pratense* L.) in New Jersey (6). *C. xenoplax* was reported (13) as the causal agent of a disease of carnation (*Dianthus caryophyllus* L.), but D. J. Raski (pers. comm.) later identified the nematode as *C. curvata*. Streu et al. (16) confirmed the pathogenicity of *C. curvata* to carnation; others reported pathogenicity to cranberry (*Vaccinium macrocarpon* Ait.) (1) and peach (*Prunus persica* [L.] Batsch) (4).

Initial experimentation indicated that, under some conditions, *C. curvata* was a weak ectoparasite of alfalfa roots. Therefore, a study was conducted to determine the pathogenicity of *C. curvata* to alfalfa and to elucidate the effects of temperature on disease and varietal reaction to *C. curvata*.

Materials and Methods

General: Gravid *C. curvata* females extracted from infested alfalfa field soil near Manteca, California, were placed in the rhizosphere of 'Moapa 69' alfalfa grown in sand-filled pots in a greenhouse. Inoculum for experiments was obtained from these cultures by centrifugal-flotation (5).

Experimental plants were grown in a heat-treated sandy loam (78% sand, 14% silt, and 8% clay). A 7% N, 6% P_2O_5, and 19% K_2O fertilizer noninjurious to *Criconemella* in greenhouse cultures (12) was applied weekly.

Plants were harvested when the first bloom appeared on any plant after the initiation of an experiment or after a prior cutting. The timing of harvests and 5-cm stubble height allowed maximum regrowth and storage of root reserves, enabling the harvest of tops to occur at approximately the same physiological age (14). All yields were based on fresh weight. At the end of each experiment, soil was removed from the root systems and passed through a 4-mm-pore sieve to collect small roots. All root material was washed, blotted dry, and weighed. Tap root diameters were measured with a micrometer. Nematode populations extracted from soil and root washings by centrifugal-flotation were determined by averaging counts from three aliquots.

Effect of inoculum potential: Seedlings of Moapa 69 alfalfa growing in 3.3-liter pots were thinned to five per pot 1 week after emergence, and 1,000 or 10,000 *C. curvata* were added to each pot; noninfested pots served as controls. In addition to a complete control, the supernatant from a suspension equivalent to the 10,000 inoculum level was included to determine growth responses from micro-organisms associated with the nematode inoculum. The supernatant was prepared by allowing the nematodes to settle overnight and removing the few remaining *C. curvata* with a nematode pick. Pots were arranged in a randomized complete block design with 10 replications. Pots were placed in sawdust beds in a greenhouse during the summer and on greenhouse benches during the winter. Supplemental incandescent lighting of 54 $\mu E\ s^{-1}\ m^{-2}$ (measured at the soil surface) with a 13-hour photoperiod was supplied from November to March. Six cuttings were harvested during the 9-month experimental period. Because of heterogeneity of variances, data were transformed to $\log_{10}(X + 1)$. An analysis of variance was conducted, degrees of freedom subtracted, and the missing plot formula applied where necessary (2). The treatment sum of squares was partitioned into single degree of freedom comparisons (15).

Upon termination of the experiment, roots were cultured for fungi. Root sections were placed in 0.5% NaOCl for 20 seconds, rinsed in sterile water, blotted dry, and cultured on 2% water agar, potato dextrose (PDA), PDA acidified with lactic acid, or a medium selective for *Pythium* and *Phytophthora* (11).

Feeding site: The feeding site of *C. curvata* was determined by growing Moapa 69 seedlings in a sand medium in petri dishes. Nematodes were added to each dish, and after 5 days hot lactophenol–acid fuchsin was poured into the dish to kill nematodes in situ at their feeding sites (9). Plants infected for 9 months were removed from pots and each root ball immersed in boiling water for 2 minutes and agitated to remove the soil. Roots were then stained in lactophenol–acid fuchsin prior to examination.

Effect of temperature: A two-factor factorial experiment was conducted in controlled temperature tanks using 0 or 4,000 *C. curvata* per pot at 22 or 27 C. Nematodes were added to 1.2-liter pots at seeding, and seedlings thinned to four per pot 2 weeks

TABLE 1. Growth of 'Moapa 69' alfalfa inoculated with *Criconemella curvata*, and increase of nematode populations during a 9-month experimental period.

Treatment	Fresh weight (g)† Shoots‡	Roots	Total plant§	Tap root diameter (mm)†	Final numbers of nematodes/pot (1,000s)†
Complete control (CC)	234.0 ± 14.3	74.0 ± 4.9	341.5 ± 20.3	7.2 ± 0.3	0 ± 0
Supernatant from 10,000 *C. curvata*	237.8 ± 12.6	77.2 ± 4.6	350.2 ± 17.6	7.4 ± 0.2	38 ± 16
1,000 *C. curvata*	201.8 ± 11.0	59.9 ± 6.5	291.8 ± 18.4	6.7 ± 0.1	1,600 ± 397
10,000 *C. curvata*	202.0 ± 11.8	56.8 ± 2.5	286.5 ± 13.3	6.5 ± 0.2	2,500 ± 344‖

Analysis of variance:

Source of variation	df	Mean squares: Weight: Shoots	Roots	Total plant	Tap root diameter
Blocks	9	774.26	0.00887	0.00246	0.17
Treatments	3	3,866.68	0.04399*	0.02041	1.52*
Nemas vs. no nemas	1	11,526.03*	0.12757**	0.06025**	4.21**
1,000 vs. 10,000	1	0.29	0.00002	0.00007	0.24
CC vs. supernatant	1	73.73	0.00438	0.00091	0.12
Error	27	1,813.61	0.01380	0.00710	0.36

† Mean and standard error of 10 replications.
‡ Total of six cuttings.
§ Total of six cuttings, stubble, and roots.
‖ $FLSD_{0.05}$ = 860 between populations resulting from addition of 1,000 nematodes, 10,000 nematodes, or supernatant.
* $P < 0.05$.
** $P < 0.01$.

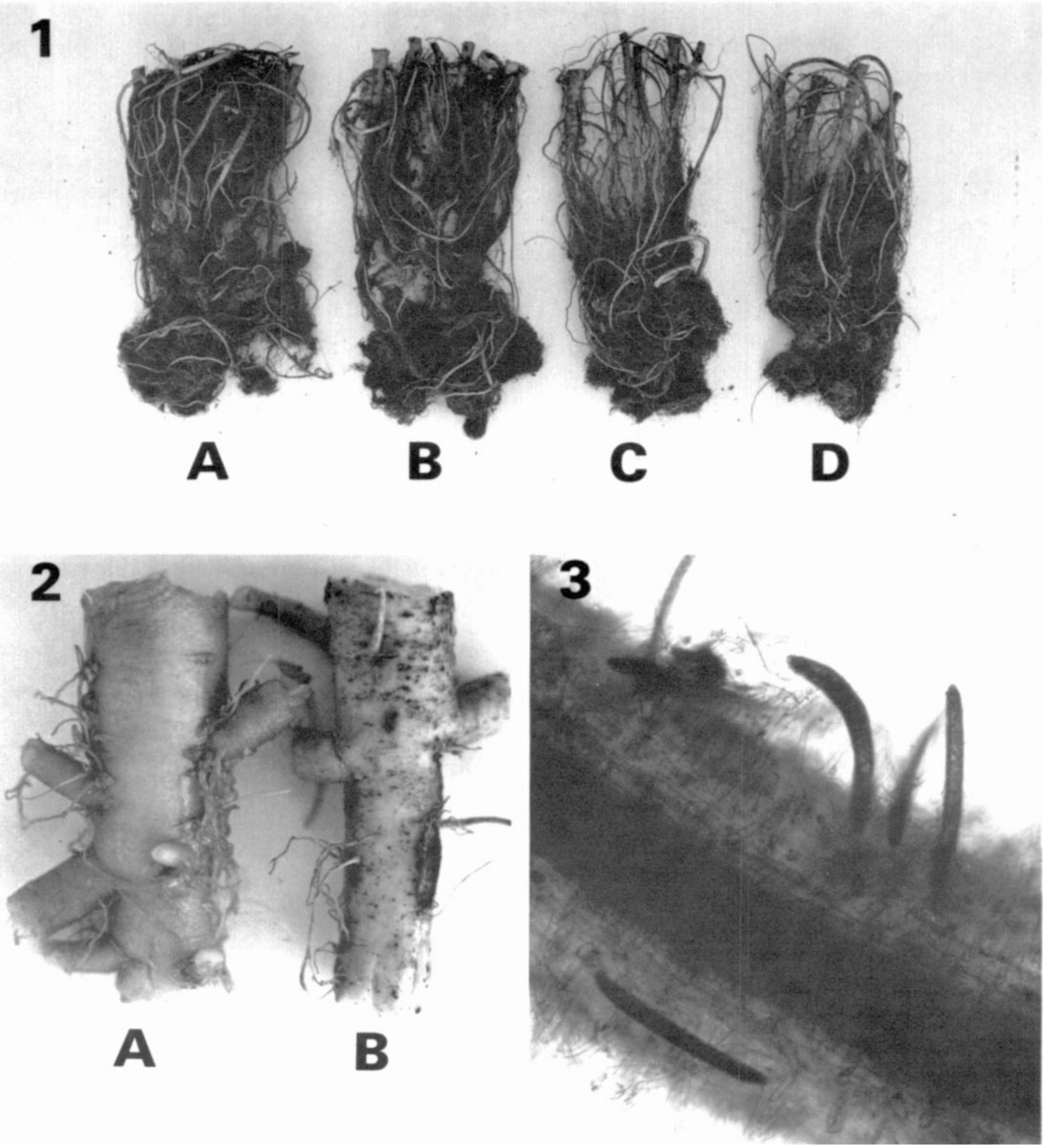
later. Treatments were replicated six times. Fresh weights from four cuttings were obtained during the 4-month experimental period.

Cultivar reaction: Either 0 or 4,000 *C. curvata* were added to 5-week-old alfalfa seedlings growing in 1.2-liter pots. The cultivars and lines included were Moapa 69, 'Lahontan,' 'El Unico,' 'Nevada Synthetic YY,' 'Nevada Synthetic XX,' 'UC-PX 1971,' and 'UC Cargo.' Treatments were replicated eight times and arranged in a randomized complete block design on greenhouse benches. Shoot growth was harvested four times, and the experiment was terminated after 6 months.

RESULTS

Effect of inoculum potential: Orthogonal comparisons showed that the 1,000 and 10,000 *C. curvata* treatments caused shoot, root, and total plant weights and tap root diameters to be different from those of the complete and supernatant controls (Table 1). No differences were observed between the two controls or between the two nematode treatments. At the end of the 9-month experimental period, numbers of nematodes in the 1,000 and 10,000 *C. curvata* treatments increased 1,600- and 250-fold, respectively (Table 1). In addition to the reduction in tap root size, there was a reduction in number of feeder roots (Fig. 1). Lesions were observed on tap and secondary roots of *C. curvata*-infected plants (Fig. 2). Differences in tap root diameters were readily visible (Fig. 2). Small populations of *C. curvata* were present in all but one experimental unit of the supernatant treatment. Nematodes which had settled out of the supernatant were added inadvertently to the plants and increased but did not reach populations large enough to influence alfalfa growth.

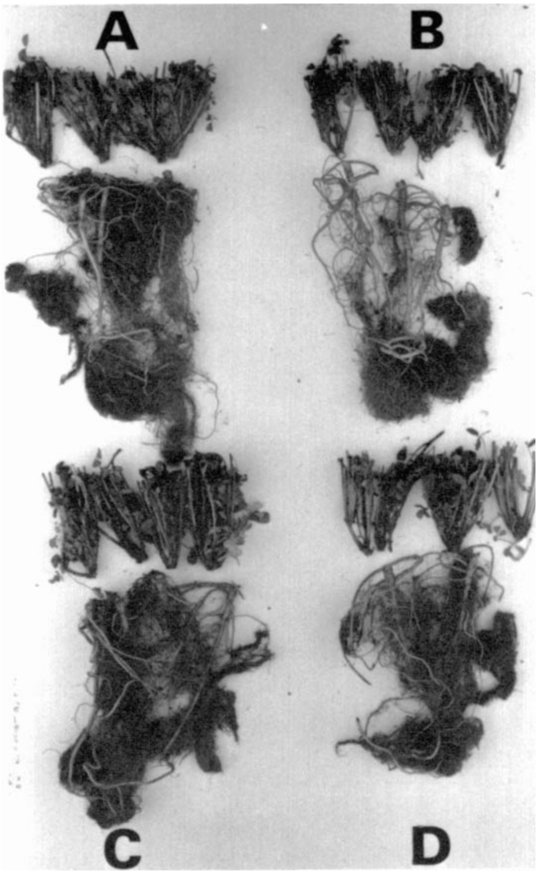
Fungi isolated from the root systems of plants in all treatments were *Alternaria alternata* (Fr.) Keissler, *Fusarium solani* (Mart.) Appel & Wr. emend. Snyd. & Hans., *Trichoderma viridae* Pers., and *Penicillium* spp. A gram-negative bacterium tentatively identified as *Rhizobium melitoti* Dang isolated from water in the nematode suspension, cultured on yeast–dextrose–calcium carbonate slants, and added to alfalfa seedlings did not affect their growth.



FIGS. 1–3. 1) Roots of Moapa 69 alfalfa 9 months after inoculation with *Criconemella curvata*. A) Complete control. B) Supernatant from 10,000 *C. curvata*. C) 1,000 *C. curvata*. D) 10,000 *C. curvata*. 2) Lesions associated with *Criconemella curvata* infection of Moapa 69 alfalfa roots 9 months after inoculation. A) Complete control. B) 10,000 *C. curvata*. 3) *Criconemella curvata* killed in situ on the tap root of a Moapa 69 alfalfa seedling.

Feeding site: Nematodes killed in situ on seedling roots occurred primarily in the zone of differentiation, but others were observed occasionally in the zone of elongation (Fig. 3). Few nematodes adhered to roots of 9-month-old plants; most were washed off when the soil was removed.

Effect of temperature: Shoot, root, and total plant weights and tap root diameters were reduced by *C. curvata*, and these interactions were unaffected by temperatures of 22 vs. 27 C (Table 2). The number of feeder roots was also reduced by *C. curvata* (Fig. 4). Numbers of nematodes increased 40-fold and 63-fold at 22 and 27 C, respectively (Table 2). In addition to the fungi found in the inoculum potential experiment, an unidentified *Phoma* sp. (not



P. medicaginis) was isolated from the roots of a nematode-infected plant grown at 27 C.

Cultivar reaction: After 6 months growth, the plants had been cut four times. There were no differences between nematode-infected and noninfected plants, including Moapa 69 which *C. curvata* had stunted in earlier experiments. The number of *C. curvata* recovered from an inoculated pot and planted to Moapa 69 was 84,000, less than one-half the population recovered in the temperature experiment which was conducted for only four months (Table 2).

Discussion

Large populations of *C. curvata* were required to adversely affect growth of alfalfa. Malek and Jenkins (8) observed no decrease in growth of hairy vetch (*Vicia villosa* Roth.), although the numbers of *C. curvata*

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Fig. 4. Roots and stubble of 'Moapa 69' alfalfa 4 months after inoculation with *Criconemella curvata* and growth at 27 C or 22 C. A) 27 C control. B) 27 C plus 4,500 *C. curvata.* C) 22 C control. D) 22 C plus 4,500 *C. curvata.*

Table 2. Effect of soil temperature on growth of 'Moapa 69' alfalfa inoculated with *Criconemella curvata* and population increases of the nematode after 4 months.

Treatment	Fresh weight (g)†			Tap root diameter (mm)†	Final numbers of nematodes/ pot (1,000s)†
	Shoots‡	Roots	Total plant§		
22 C noninfected	82.9 ± 1.5	26.5 ± 2.2	119.6 ± 4.4	5.3 ± 0.2	0 ± 0
22 C infected	66.6 ± 3.2	19.7 ± 1.3	95.2 ± 4.0	4.8 ± 0.2	181 ± 25
27 C noninfected	91.4 ± 5.5	24.1 ± 1.5	126.4 ± 6.4	5.3 ± 0.3	0 ± 0
27 C infected	72.6 ± 3.6	17.6 ± 1.5	98.6 ± 5.2	4.6 ± 0.3	284 ± 48‖

Analysis of variance:

Source of variation	df	Mean squares: Weight: Shoots	Roots	Total plant	Tap root diameter
Treatments	3	0.0225**	99.21**	1,415.12**	0.79*
A = *C. curvata*	1	0.0580**	266.66**	4,070.61**	2.28**
B = temperature	1	0.0094	30.82	157.08	0.04
Interaction A × B	1	0.0002	0.15	16.67	0.06
Error	20	0.0024	16.53	154.49	0.22

† Mean and standard error of six replications.
‡ Total of four cuttings.
§ Total of four cuttings, stubble, and roots.
‖ Differs from the number of *C. curvata* at 22 C at $P < 0.05$ according to Student's *t*-test.
* $P < 0.05$.
** $P < 0.01$.

increased from 1,000 to 56,000 in 3 months. *C. curvata* reduced alfalfa growth in two of the three experiments reported here. The anomalous experiment was the test of varietal reaction which was conducted during the summer. Although soil temperature was ameliorated by shade and cooling, 30 C was exceeded frequently for many hours each day. Malek and Jenkins (8) reported greater reproduction of *C. curvata* at 25 C than at 15, 20, or 30 C. The greater increase in nematode population at 27 C than at 22 C which we observed corroborates the observations of Malek and Jenkins (8). Temperature probably had a direct effect on the nematode rather than an indirect effect related to food supply, since temperature did not affect alfalfa root weights significantly.

Fungi isolated from the alfalfa root systems in the inoculum potential and temperature experiments are cosmopolitan and were probably aerial or water-borne contaminants since they occurred in all treatments.

The root lesions associated with *C. curvata* feeding resemble those reported on banana (*Musa* sp.) caused by *Helicotylenchus multicinctus* (Cobb) Thorne (7), boxwood (*Buxus sempervirens* L.) caused by *Rotylenchus buxophilus* Golden (3), and *Lespedeza cuneata* (Dumont) G. Don caused by *Hoplolaimus tylenchiformis* Daday (10). No lesions were reported on *C. curvata*-infected carnation (13,16) where nematodes fed on root tips, in the zone of differentiation, and on older roots. In alfalfa the lesions extended only a few cells into the cortex of the tap root and secondary roots. On seedlings nematodes fed primarily in the zone of differentiation, but also in the region of elongation. Nematode feeding reduced the number of feeder roots. Lesions on tap roots and secondary roots indicated that the nematode fed in these areas also, although few nematodes were killed in situ on these roots; most nematodes were removed along with soil particles when the roots were washed.

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