The Effects of Ammonia-Generating Fertilizer on Criconemoides xenoplax in Pot Cultures[1]

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Abstract: Fertilizer which generated NH_3 was detrimental to *Criconemoides xenoplax* grown in the greenhouse. The fertilizer was lethal to the nematodes *in vitro* only when it was accompanied by urease-positive bacteria or partially purified urease. The detrimental action of the fertilizer-urease mixture was more rapid at pH 8 than at pH 7. *Key Words:* Ring nematodes, urea.

Myrobalan and Marianna plum selections are known to be excellent hosts for the ring nematode, *Criconemoides xenoplax* Raski (8). In one experiment, however, we had difficulty in increasing this nematode on 'Myrobalan' (*Prunus cerasifera* Ehrh.) seedlings and on plants grown from 'Marianna 2624' *Prunus cerasifera* Ehrh. x *P. munsoniana* Wight & Hedr. cuttings. It was important to determine the cause of the difficulty so that it could be avoided in future experiments.

The kind of fertilizer used in our experiments varied. In the experiment in which we had difficulty, we used Chevron Chemical Company's Ortho Gro® liquid plant food containing 12% N in the form of urea, 6% P, 6% K, and 4% EDTA. Plant growth resulting from use of this fertilizer was excellent, but we questioned whether NH_3 or nitrite resulting from decomposition of this fertilizer's urea constituent (1, 13) might be affecting *C. xenoplax* adversely. Ammonia and nitrite have been associated with reduction in populations of other plant-parasitic nematodes (12). Greenhouse and laboratory tests were used to test the possibility.

MATERIALS AND METHODS

Greenhouse: Myrobalan and Marianna 2624 plants (growing singly in 20-cm clay pots) were inoculated with 1,500 *C. xenoplax* and fertilized with Ortho Gro liquid plant food at the suggested rate (4,000 μ liter/liter), at one-half and twice this concentration, and at several frequencies of application (Tables 1, 2). To prevent poor growth of the host plant from limiting nematode reproduction,

TABLE 1. Influence of fertilizer amendments on reproduction of *Criconemoides xenoplax* on Marianna 2624 plum.

Fertilizer treatment	Nematode numbers/pot (time after inoculation—months)[a]		
	1	2	3
Hyponex (300 ml monthly)			
1,300 μg/ml	1,319x	7,314x	47,633x
Ortho Gro (300 ml weekly)			
2,000 μliter/liter	345x	60y	44y
4,000 μliter/liter	331x	147y	2y
8,000 μliter/liter	289x	28y	0y

[a]Average of six replicates; averages not followed by the same letter in each time period differ according to Student's t test ($P = <0.01$). The number of *C. xenoplax* added initially was 1,500/pot.

TABLE 2. Effects of fertilizer regimes on the growth of Myrobalan plum and reproduction of *Criconemoides xenoplax*.[a]

	Root wt (gm)	*C. xenoplax*/ gm of root
Hyponex (1,300 μg/ml)		
300 ml monthly	32.8x	1,028w
Ortho Gro		
(4,000 μliter/liter)		
300 ml monthly	47.3x	26x
300 ml every 2 weeks	29.3xy	1y
300 ml weekly	19.7y	0y

[a]Average of six replicates 3 months after inoculation; averages not followed by the same letter, within a column, differ according to Student's t test ($P = <0.05$). The number of *C. xenoplax* added initially was 1,500/pot.

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Hydroponic Chemical Company's Hyponex® was used instead of a nontreated control. This fertilizer contains 7% N, 6% P, and 19% K. The N is largely in the form of nitrate; thus little NH_3 would be generated from it. Centrifugal flotation (5) was used to obtain nematode inoculum and to assay nematode population levels. For the assay, the soil in each pot was mixed to homogeneity before nematodes were extracted from an aliquant. In addition, each root system was agitated in water and the nematodes in an aliquot of this water were counted. After reproduction, the nematodes from soil and roots were combined to obtain the total number of *C. xenoplax* per pot. At the end of one of the greenhouse experiments, soil samples from each pot were analyzed for nitrite (2).

Treatments were arranged in randomized blocks and Student's t test was used to compare each treatment mean with other treatment means.

Laboratory: Criconemoides xenoplax was incubated in distilled water and in a liquid basal medium to which Ortho Gro liquid plant food or its constituents (with or without urease or urease-producing bacteria) were added. The sterile basal medium (pH 7.4) contained casein hydrolyzate—100 μg/ml, glucose—1,000 μg/ml, K_2HPO_4—400 μg/ml, and NaCl—250 μg/ml. In two experiments (Tables 4, 5), 0.05 M phosphate buffer was used to maintain a constant pH, and phosphate-buffer controls were included. Nematodes were extracted (5), and washed once with a solution (2,000 μliter/liter) of Zephiran chloride (benzalkonium chloride 17%) and three times with a solution (8,000 μg/ml) of 74% dihydrostreptomycin sulfate. Centrifugation was used to separate the nematodes after each wash. They were then shaken for 6 h in the dihydrostreptomycin sulfate and washed 3 times in sterile water. A drop of the nematode suspension treated in this manner was placed on urea agar (7) and found to be free of urease-positive bacteria. The urease-positive bacteria, which were added in some treatments, were isolated by the dilution method (6) from soil around experimental Myrobalan plum seedlings infested with *C. xenoplax*. A urease-positive strain was selected on the basis of its response to urea agar (7). Tests involving this bacterium were made in a basal liquid medium (Table 3) which supplied essential nutrients but was sufficiently dilute to be harmless to the nematodes over the 48-h duration of the test. Movement after touch stimulus with a fine nylon pick was used as the criterion for nematode viability. Arcsin transformation of data corrected heterogeneity of error before analysis of variance. Duncan's Multiple range test was used to separate means.

TABLE 3. Survival of *Criconemoides xenoplax* exposed to a basal medium with different nitrogen sources, and with or without urease-positive bacteria (UPB).

Nitrogen source	Percent survival[a]
Distilled water control	93x
KNO_3 1,800 μg/ml	80x
KNO_3 1,800 μg/ml + UPB	92x
Ortho Gro 8,000 μliter/liter	85x
Ortho Gro 8,000 μliter/liter + UPB	23y
Urea 940 μg/ml	92x
Urea 940 μg/ml + UPB	31y

[a]Average of three replicates after 48-h exposure. Averages not followed by the same letter differ according to Duncan's multiple range test ($P = <0.01$).

RESULTS AND DISCUSSION

Use of Ortho Gro liquid plant food as a fertilizer for Marianna 2624 and Myrobalan cultivars infected with *C. xenoplax* suppressed nematode reproduction and caused populations to decline (Tables 1, 2). The detrimental effect increased with the number of applications and with concentration of the fertilizer. Host plants were more tolerant of this fertilizer than were the nematodes, but the plants were adversely affected by the highest rates of application and concentration (Table 2). Marianna 2624 was less seriously affected than Myrobalan, probably because it was more vigorous and exhausted the fertilizer more rapidly. The nitrite content of the soil was not altered by these fertilizer applications.

In short-term *in vitro* laboratory tests, neither Ortho Gro nor its major constituents affected the viability of *C. xenoplax* (Tables 3, 4, 5). However, when this fertilizer or its urea constitutent was

TABLE 4. Percent of *Criconemoides xenoplax* surviving each of three successive 6-hour exposures to chemical treatments.

Treatment	Percentage (%) and pH/exposure time[a]					
	6 h		12 h		18 h	
	%	pH	%	pH	%	pH
Distilled-water control	91w	6.5	84w	6.9	90w	7.0
0.05-M phosphate-buffer control	80w	7.0	88w	7.0	73w	7.1
Urease 3 units[b]	75w	7.0	98w	7.0	88w	7.0
Urease 6 units	77w	7.0	69w	7.0	35x	7.0
Ortho Gro 4,000 μliter/liter	88w	7.0	85w	7.1	100w	7.1
Ortho Gro 8,000 μliter/liter	73w	7.1	83w	7.1	95w	7.1
Ortho Gro 4,000 μliter/liter + 3 units urease	40x	7.9	38x	7.8	15y	8.0
Ortho Gro 8,000 μliter/liter + 6 units urease	2y	8.4	0y	8.5	—	—
Urea 470 μg/ml	78w	7.0	83w	7.0	90w	7.0
Urea 940 μg/ml	81w	7.0	85w	7.0	73w	7.0
Urea 470 μg/ml + 3 units urease	80w	7.3	77w	7.4	3z	7.4
Urea 940 μg/ml + 6 units urease	52x	7.8	50x	7.8	0z	7.8

[a]Average of four replicates. Averages not followed by the same letter in each row or column differ according to Duncan's multiple range test ($P = <0.01$). One pH measurement made for each treatment, at the end of the treatment.

[b]One unit is the amount of enzyme which will produce 1 mg of ammonia nitrogen from urea in 5 min at pH 7 and 30 C.

present with urease or urease-producing bacteria in sufficient concentrations, many nematodes were killed (Tables 3, 4, 5). Urease-producing bacteria increased in all treatments to which they were added and reached a final concentration of 2 x 10^7 cells/ml after 48 h. Urease facilitates the conversion of urea to NH_3 and CO_2. The odor of NH_3 was detected whenever urease was present with high concentrations of Ortho Gro or urea. Ammonia is known to be toxic to nematodes (4, 10).

Final measurements of pH suggest that one effect of the ammonia-generating mixtures may have been to increase alkalinity (Tables 4, 5). Buffer solution adjusted to pH 9 did not kill *C. xenoplax* (Table 5). High pH, therefore, was probably not the direct cause of nematode death. Examination of nematodes incubated in the various solutions revealed no loss in turgidity which might accompany osmotic effects. The suppression of populations of *C. xenoplax*, which was associated with use of Ortho Gro in pot cultures, is attributable to the NH_3 generated from the urea constituent of this fertilizer.

Suppression of nematode populations by urea has been observed repeatedly (3, 9, 11, 12). For several reasons, urea has not been a very efficient or consistent nematicide under field conditions. It is highly water soluble and, in areas of high rainfall, can be washed away before it is converted to the actual toxicant NH_3 (11). Also, the amount of NH_3 occurring in equilibrium with other forms of ammoniacal nitrogen is a function of pH (13). NH_4^+ (ion) accumulation is favored by low pH, and urea is not an effective nematicide in soils whose pH is low. Probably most important is the fact that the rate of diffusion of NH_3 in soil is low (4), and it is nematicidal only in a limited area near the point of its application or generation. The dosage required for nematode control in the field may be phytotoxic (3).

In the case of container-grown plants, however, the limited volume of soil may be permeated by NH_3. An ectoparasitic nematode (such as *C. xenoplax*) has no protection from it. Caution is suggested in use of fertilizers containing urea as nutrients for plants used in culture of nematodes in containers.

TABLE 5. Percent of *Criconemoides xenoplax* surviving 6 hours' exposure to ammonia-generating compounds.

Treatments	Percent survival[a]	pH[b]
Distilled-water control	97x	6.9
0.05-M phosphate-buffer control	90x	8.0
0.05-M phosphate-buffer +KOH control	85x	9.0
Urease 3 units[c]	81x	8.0
Urease 6 units	89x	8.0
Ortho Gro 4,000 μliter/liter	97x	7.9
Ortho Gro 8,000 μliter/liter	86x	7.9
Ortho Gro 4,000 μliter/liter + 3 units urease	0y	8.9
Ortho Gro 8,000 μliter/liter + 6 units urease	0y	8.9
Urea 470 μg/ml	90x	8.0
Urea 940 μg/ml	83x	8.0
Urea 23 μg/ml + urease 0.15 units	86x	8.0
Urea 47 μg/ml + urease 0.30 units	86x	8.0
Urea 94 μg/ml + urease 0.60 units	85x	8.1
Urea 470 μg/ml + urease 3.00 units	0.003y	8.8
Urea 940 μg/ml + urease 6.00 units	0y	8.9

[a]Average of four replicates. Averages not followed by the same letter differ according to Duncan's multiple range test ($P = <0.01$).
[b]One pH measurement made for each treatment at the end of the treatment.
[c]One unit is the amount of enzyme which will produce 1 mg of ammonia nitrogen from urea in 5 min at pH 7 and 30 C.

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