Effects of Aldicarb and Its Sulfoxide and Sulfone on the Biology of Tylenchulus semipenetrans

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Abstract: In laboratory testing, egg hatch of *Tylenchulus semipenetrans* was stimulated at concentrations of 1 and 10 μg/ml aldicarb solution and inhibited at 50 and 100 μg/ml. Aldicarb was more inhibitory to egg hatch than the aldicarb sulfoxide and the aldicarb sulfone. Inhibition of hatch at the high concentration was associated with delays in the molting processes, lack of larval movement within the egg, and delays in embryonic development. Nematode motility was reduced at 10, 50, and 100 μg/ml of aldicarb and aldicarb sulfoxide solution, and at 50 and 100 μg/ml aldicarb sulfone. Male development was retarded at 10 μg/ml and almost completely inhibited at 50 and 100 μg/ml of the three chemicals. In greenhouse tests, female development and reproduction on roots of citrus seedlings were suppressed by aldicarb at rates of 2.6 μg/ml and completely inhibited at 10.6 μg/ml of soil solution during a 50-day experimental period. Under field conditions, there was little systemic movement of aldicarb into roots located outside treated areas. Aldicarb reduced the nematode larvae and the female adult population in the second year after the second treatment. There were no differences in egg hatch and sex ratio of citrus nematodes between treated and nontreated roots. *Key Words:* citrus nematode, hatching, development, reproduction, chemical control, nonfumigant nematicide, motility, mortality, mode of action.

Aldicarb has been tested in field situations for control of the citrus nematode (*Tylenchulus semipenetrans* Cobb) and for some foliar feeding insects (1), (Baines and Carmen, personal communication). It may have potential for use in an integrated pest control program on citrus because of systemic insecticidal properties, low phytotoxicity, ease of application, and narrow biological spectrum (7). The mode of action of aldicarb in insects is primarily as a cholinesterase inhibitor (17), and it is suspected that the mode of action in plant-parasitic nematodes is the same (14, 19). Symptoms of toxicity include disruption of male orientation (5), inhibition of egg hatch (5), nematode invasion (16), and nematode development (15). In the plant and probably within the nematode, aldicarb is rapidly metabolized—primarily to aldicarb sulfoxide and, to a lesser extent, to aldicarb sulfone (17). In soils, aldicarb is very transient (3, 6, 15). Aldicarb sulfoxide is formed rapidly and eventually converted by hydrolysis to nontoxic products. About 10% of the original aldicarb is converted to the sulfone. The toxicity of aldicarb sulfoxide to animals is unchanged from aldicarb; however, the toxicity of aldicarb sulfone is considerably lower to mammals (17), insects (17), and possibly nematodes (9, 15).

The purpose of this research was to compare the toxicity of aldicarb with the toxicity of two soil degradation products, the sulfoxide and the sulfone, on the behavior and biology of *T. semipenetrans.* Because of the complexity of the life cycle of this nematode, tests were conducted in the laboratory, greenhouse, and in citrus orchards.

MATERIALS AND METHODS

Egg extraction: Large quantities of eggs of *T. semipenetrans* were collected from infected citrus roots by gently washing, cutting into 1- to 2-cm long pieces, and blending in 100 ml of water for 10 seconds. The liquid and plant material were then poured through 88.9-μm (170-mesh) and 25.4-μm (500-mesh) screens. The plant material collected on the 88.9-μm screen was rinsed to wash all remaining loose eggs onto the 25.4-μm screen. The resulting mixture of eggs, larvae, and female citrus nematodes was washed from the 25.4-μm screen into a beaker. Final egg extraction was achieved by again passing the mixture two or three times through 88.9-μm screens so that eggs could be collected while larvae were allowed to pass through the screen. Only a few larvae remained in the final collection beaker.

Newly hatched larvae preparation: Freshly hatched nematode larvae were ob-

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tained from infected citrus roots aerated in water. Newly hatched larvae were collected every 24 h by passing the water from the roots through 88.9- and 25.4-μm screens. The larvae caught on the 25.4-μm screen were transferred to Baermann funnels to separate active larvae. Active larvae were stored at 4 C until tested. Maximum age of larvae used in any experiment was 14 days; however, in most experiments it was 3 days.

Chemicals: Technical grade aldicarb, aldicarb sulfoxide, and aldicarb sulfone (Union Carbide Corporation) were used in all laboratory experiments. Distilled water (pH 6.8-7.4) was used to prepare solutions. Granules of aldicarb (15 G) were used in greenhouse and citrus-orchard tests. In the greenhouse tests, aldicarb (15 G) granules were blended in water for 5 min, and the chemical solution recovered by the suspension being poured over a 149.8-μm (100-mesh) screen to hold back the corn-cob grit. The residue was collected and blended again to extract any remaining chemical.

RESULTS

Embryonic development of egg hatch: Equal volumes (7.5 ml) each of egg suspensions and chemical solution were mixed in test tubes to make final concentrations of 1, 10, 50, and 100 μg/ml. The final suspension in each tube was 15 ml. The test tubes were incubated at 27 C. The chemical solution was removed daily and replaced with the respective freshly prepared chemical solution to avoid aeration problems and degradation of the original chemical. Eggs and newly hatched larvae in each tube were collected on a 25.4-μm screen after 18 days and resuspended in distilled water. Each suspension was divided into two portions. One portion was incubated for 14 days at 27 C with daily changes in distilled water and then examined for hatched larvae. The other portion was fixed in warm 5% formalin and the number of embryos, larvae within eggs, and hatched larvae were counted in each sample. The experiment was repeated with 3 and 5 replications in each treatment.

The normal development of *T. semipenetrans* from embryos into first-stage larvae within the eggs was nearly complete within 8 days in distilled water, and approximately 48% of all eggs hatched in 18 days. There was a stimulation in the

TABLE 1. Percent embryos, unhatched larvae in eggs, and hatched larvae of *Tylenchulus semipenetrans* exposed to distilled water, aldicarb, aldicarb sulfoxide, and aldicarb sulfone.

Treatments	Concentrations (μg/ml)	Chemical solution 18 days[a]			Water 14 days[a]
		Embryos %	Larvae in eggs %	Hatched larvae %	Hatched larvae %
Water control		48	4	48	67
Aldicarb	1	23*	10	67*	67
	10	27*	11	62	63
	50	58	23	19*	39*
	100	54	42*	4*	34*
Aldicarb sulfoxide	1	32	5	63	64
	10	31	5	64	69
	50	30	23	47	54
	100	39	26*	35*	46
Aldicarb sulfone	1	39	6	55	55
	10	30	9	61	61
	50	42	10	48	59
	100	50	17	33*	43

[a]Asterisk (*) indicates significant difference from control ($P=0.05$). Eggs and larvae remained in chemical solution for 18 days and were then placed in distilled water for 14 days.

hatching of eggs at 1 μg/ml aldicarb. Hatching was inhibited by 29 and 44% when eggs were exposed to 50 and 100 μg/ml aldicarb, respectively. Aldicarb sulfoxide, and aldicarb sulfone at 100 μg/ml, inhibited egg hatch by 13 and 15%, respectively. From 24 to 58% of the larvae in eggs that had been treated with 50 and 100 μg/ml of the three chemicals for 18 days hatched during an additional 14 days in distilled water. Aldicarb at 100 μg/ml was the most toxic of the three chemicals and suppressed hatching more than either the sulfoxide or sulfone (P@ 0.05).

Motility: Tests for nematode motility were conducted in 20-ml brown bottles using the Moje technique (8). Suspensions of newly hatched larvae in 3.5 ml water were placed in bottles; each contained 3.5 ml aldicarb, aldicarb sulfoxide, or aldicarb sulfone solutions at concentrations appropriate for making the final concentrations of 1, 10, 50, and 100 μg/ml. Nematodes exposed to 7 ml distilled water served as the control. The bottles with filters were turned upside down for 24 h into 26-ml petri dishes containing 12 ml of respective chemical solution. Nematode motility was determined by counting the nematodes that migrated into each petri dish. There were two trials with 4 replications in each treatment.

Aldicarb, aldicarb sulfone, and aldicarb sulfoxide at 1 μg/ml did not reduce nematode motility as compared to the check (Fig. 1). Nematode motility was reduced (P@0.05) in the 10 μg/ml aldicarb and aldicarb sulfoxide solutions but not in the aldicarb sulfone solution.

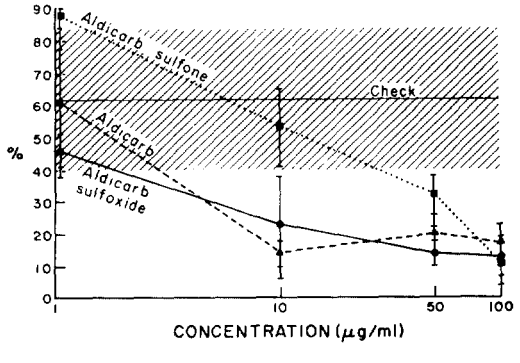

FIG. 1. Motility of *Tylenchulus semipenetrans* larvae treated with three chemicals at concentrations of 0, 10, 50, and 100 μg/ml for 24 h and then allowed to migrate through a screen bathed in the same concentration for 24 h. Total exposure to chemical was 48 h at 27 ± 1 C. Slash marks indicate variability (P@ 0.05).

Male development: The male larva of *T. semipenetrans* can develop into an adult in water without feeding, and the different larval stages can be distinguished by the body morphology and the remaining intact molt cuticles (18). The methods for testing the effects of the three chemicals on male development were similar to those described for testing egg hatch except larval samples were removed from each chemical solution every 24 h for 8 days. The stage of male development was determined under a compound microscope (120X). The percentage of each stage was recorded for a random sample of 50-100 nematodes. There were two experiments with three and five replications.

Concentrations of aldicarb and its sulfoxide or sulfone of 50 and 100 μg/ml inhibited the development of male larvae from second-stage to third, fourth, and adult males (Table 2). Larval development to adult males was reduced at 10 μg/ml of all three chemicals. There was no inhibition at 1 μg/ml. The three chemicals also in-

TABLE 2. Percent males developing into the next advanced stage within 8 days at concentrations of chemicals tested.

Treatments	Concentration (μg/ml)	Nematode stages III %	IV %	Adult %
Water Control		100	97	39
Aldicarb	1	100	97	38
	10	86	50	12
	50	19	0	0
	100	12	0	0
Aldicarb sulfoxide	1	—[a]	—	—
	10	87	51	13
	50	57	0	0
	100	28	0	0
Aldicarb sulfone	1	100	92	41
	10	97	74	17
	50	45	5	2
	100	6	0	0

[a]Minus (—) no data obtained.

duced morphological aberrations, including wrinkling, shortening, and swelling of the body of male larvae (Fig. 2). All three chemicals were equally effective in their inhibition of male development.

Female development and reproduction: The development of female larvae was studied on "Homosassa" sweet orange (*Citrus sinensis* L.) seedlings (10-month-old, 30-40 cm high) grown in sandy-loam soil in 1-liter plastic pots at 27 C. The water content of soil in the pot was maintained at about 25% (on the basis of soil dry weight calculated at 100 C for 8 h). The seedlings were inoculated with about 72,000 newly hatched larvae/pot and incubated for 2 weeks prior to the application of chemical, to enable the nematode to begin feeding on roots. Aldicarb was applied at 1 and 4 μg/g of dry weight soil (solution concentrations were calculated at 2.6 μg/ml and 10.5 μg/ml soil solution, respectively) to the pots in 20 ml of water (4 replicates per treatment). Starting approximately 14 days after nematode infestation and 5 days after chemical treatment, the eggs, larvae, and female adults were collected by the same method as described for egg extraction and counted under the dissecting microscope.

The successful feeding and development of second-stage female larvae into young females was not inhibited at 2.6 μg/ml (Fig. 3); however, fewer young females developed into mature females during the 50-day experiment ($P=0.05$). Almost no development of mature females occurred within 50 days of treatment at 10.5 μg/ml. The second generation, as compared to those on roots of nontreated seedlings, was slowed and reduced in both treatments.

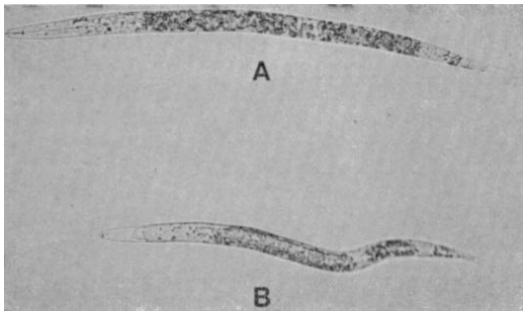

FIG. 2. The effects of aldicarb at 100 μg/ml on the molting of male larvae of *Tylenchulus semipenetrans*. A) Normal fourth-stage male larvae. B) Abnormal second-stage male larvae inhibited from molting into third-stage male larvae.

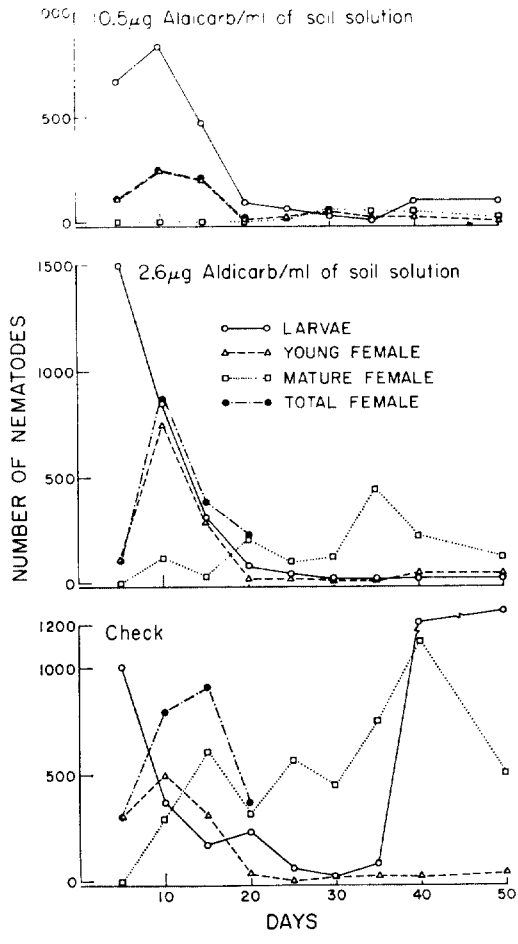

FIG. 3. The effects of aldicarb at concentrations of 0 (check), 2.6, and 10.5 μg of aldicarb/ml of soil solution on the development and reproduction of *Tylenchulus semipenetrans* on citrus seedlings growing at 27 ± 1 C.

Egg and larvae production/female were suppressed by 45% and 80%, respectively, in both aldicarb treatments 50 days after treatment.

The effects of aldicarb on female development and reproduction was also tested on field trees treated with aldicarb (15 G). One experiment was conducted on 14-year-old navel orange citrus trees on a sandy loam soil and was sampled 8 months after a second year treatment (20 months after the first treatment) with 11.2 to 22.4 kg(a.i.)/ha of aldicarb in the treatment area. The 15% granules were applied around the tree drip-line in a band 61 cm wide and rototilled to a depth of 5-8 cm. Treatment was followed by sprinkler irrigation for 24 h on the first day and two

subsequent irrigations. This site is identified as Field A. A second site of valencia orange trees planted in the same field area is referred to as Field B. All treatments were replicated 6 times. (Samples were taken from field plots established and maintained by R. C. Baines and G. E. Carmen, University of California, Riverside). The application of the granules in Field B was in a 1-m band around the dripline of the tree at a rate of 11.2 kg(a.i.)/ha in treated area which was rototilled and sprinkle irrigated. Samples were taken 2 months after the second annual treatment or 14 months after the first treatment.

Aldicarb applied annually at 11.2 to 22.4 kg/ha for 2 successive years suppressed the development of mature females, larvae, and eggs ($P=0.05$) on citrus roots located in treated soil in Fields A and B (Tables 3 and 4). Egg and larval development per female was suppressed in Field A but not in Field B.

Eggs, larvae, young females, and mature females were extracted from treated and nontreated roots and counted to determine the total nematode population of the roots. Systemic action of the chemical to the distal portions of roots located outside the treated area was determined in Field A.

Egg hatch in the treated field soil (Field B) and nontreated soil was also checked by placing soil (5 replicates) in petri dishes and adding an egg suspension of about 144,000 eggs/dish. Water contents in both soils were controlled at 21 $\pm$ 2 % soil dry weight. The hatched nematodes in soil were collected after 18 days by using the 0.5 M-sucrose flotation method (2).

Systemic activity was tested by obtaining roots not previously treated with aldicarb from the center area of four trees previously treated with aldicarb in the 61-cm band. There were no effects of aldicarb on the populations of nematodes found on nontreated roots (Table 3). Soil obtained 2

TABLE 3. Citrus nematodes found on navel citrus roots (Field A) treated with aldicarb.

Treatments	Females		Larvae		Eggs		Eggs & larvae/female	
	tree[x]	center[y]	tree	center	tree	center	tree	center
Aldicarb—treated plots[z]	403a	487a	2,902a	3,035a	8,913a	9,650a	29a	26a
Nontreated plots	827b	560a	7,406b	3,435a	25,170b	9,961a	40b	25a

[x,y]The tree sampling area is from the 61-cm band surrounding the tree. The center sampling area was from nontreated soil outside the treated band [11.2-22.4 kg(a.i.)/ha] and received no chemical except by systemic activity through roots 8 months after second application and 20 months after first.
[z]Averages of 5 replications each/g root. Two treatment means followed by the same letter do not differ from each other according to Duncan's multiple range test (P@ 0.05).

TABLE 4. Citrus nematodes found on Valencia citrus roots (Field B) treated twice with aldicarb.

Treatments	Mature females	Young females	Larvae	Eggs	Eggs & larvae/female
Aldicarb-treated plot[z]	1,180a	420a	8,600a	18,860a	24a
Nontreated plot[z]	3,480b	720b	16,280b	30,508b	17a

[z]Averages of 4 replications each. Two treatment means/g root followed by the same letter do not differ from each other according to Duncan's multiple range test (P@ 0.05) 2 months after second application and 14 months after first.

months after aldicarb treatment in the field was not effective in retarding egg hatch (Tables 3 and 4) in comparison with nontreated soil. Eggs from roots in nontreated and aldicarb-treated soil showed the same hatching rate and the same sex ratio, an indication that toxicity of aldicarb as measured biologically had disappeared in 2 months.

DISCUSSION

Past performance data of aldicarb and its sulfoxide and sulfone for nematode control have indicated that they are *nematistatic* in action rather than *nematoxic* (4, 7, 10, 12, 13, 19). The success of aldicarb as a nonfumigant nematicide appears to be more closely associated with behavioral modifications of individuals or inhibition of some particular stage in their life cycles (5, 11). Field applications of aldicarb to citrus have given some success in control of citrus nematode (1).

In controlled hatching studies, the effects of aldicarb and its sulfoxide and sulfone in solution were similar in pattern to those reported on *Heterodera schachtii* (5, 15), and *H. rostochiensis* (9). At 1 and 10 μg/ml, aldicarb stimulated embryonic development of citrus nematode and egg hatch and was inhibitory at 100 μg/ml. These results indicate that aldicarb and its two major soil degradation products are less toxic to *T. semipenetrans* than to *Heterodera* or *Meloidogyne* spp. Larval escape from the egg was inhibited, probably because of retarded movement and activity of the larvae in eggs. This suppression of hatching appears to be closely associated with effects of the chemicals on larval motility.

Probably the most significant effect of aldicarb at relatively low concentrations in solution (1-10 μg/ml) on *T. semipenetrans* was the inhibition and/or disruption of the molting process. This response was observed in hatching studies of embryos molting to complete second-stage larvae and in molting studies of second-stage male larvae to adult males. Rates of hatch and molt were suppressed at 10 μg/ml concentration of aldicarb.

Confirmation of the effects of these chemicals on the development of female larvae was more difficult and had to be conducted on citrus seedlings or mature citrus trees in a soil medium. Precise measurements of concentration and equal distribution of chemical throughout the system were obviously more variable than in laboratory tests with solutions. Variability was even greater in field applications, but the results did show that low concentrations of aldicarb and its metabolic products inhibit the development of females and the production of eggs by adults.

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