Behavioral Responses of *Heterodera rostochiensis* Larvae to Aldicarb and Its Sulfoxide and Sulfone[1]

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Abstract: Temik® aldicarb pesticide [2-Methyl-2-(methylthio) propionaldehyde-O-(methylcarbamoyl) oxime] is an effective contact and systemic compound against a wide variety of agricultural pests. Its metabolism in soils may lead to aldicarb sulfoxide and aldicarb sulfone which are both toxicologically important. The comparative effects of these compounds on body activity and stylet movement of second-stage larvae of the potato cyst nematode, *Heterodera rostochiensis,* were investigated. Temik aldicarb was the most effective contact toxicant, rapidly inhibiting body activity and stimulating abnormal stylet movement. A 24-hr post-nematicide water treatment allowed effective recovery of body vigor and cessation of abnormal stylet movement of the larvae treated with Temik aldicarb at low concentrations, and with aldicarb sulfoxide and aldicarb sulfone at all the dosage levels used. Larvae treated with 10 ppm Temik aldicarb remained paralyzed, the toxic effect being apparently irreversible. Control of *Heterodera rostochiensis* by direct contact toxicity may not be effective in soil since Temik degrades to compounds having reversible toxic effect. *Key Words: Heterodera rostochiensis,* Golden nematode, Potato cyst nematode, Aldicarb, Nematicide, 2-Methyl-2-(methylthio) propionaldehyde-O-(methylcarbamoyl)oxime, Toxicity.

Temik® (Union Carbide Corp., New York, N.Y.) aldicarb pesticide [2-Methyl-2-(methylthio) propionaldehyde O-(methylcarbamoyl)oxime] is taken up by plant roots and moves systemically. Its biological properties are interesting and it is potentially useful against a variety of agricultural pests. As an aphicide it is effective against the pea aphid (*Acyrthosiphon pisum*) on alfalfa seedlings (14) and the black bean aphid (*Aphis fabae*) on broad beans (12), and as an insecticide and acaricide against pests on ornamental plants (11). Nematicidal properties have been reported for the control of the potato cyst nematode, *Heterodera rostochiensis*, on potatoes (9) and for root-knot nematodes on kenaf (*Hibiscus cannabinus* L.) (1).

In soil Temik aldicarb degrades by oxidation or hydrolysis to a number of metabolites (3) several of which have important toxicological properties which merit investigation. Temik sulfoxide (= aldicarb sulfoxide) [2-Methyl-2-(methylsulfinyl) propionaldehyde O-(methylcarbamoyl)oxime] formed from the initial oxidation of Temik (2), is a potent cholinesterase inhibitor in insects (4), fairly persistent in soils and is rapidly translocated in the plant (8). Temik sulfone (= aldicarb sulfone) [2-Methyl-2-(methylsulfonyl) propionaldehyde O-(methylcarbamoyl)oxime] formed along the same oxidative pathway from aldicarb sulfoxide (2) is a weak cholinesterase inhibitor in insects (8).

Since investigators have shown the systemic and contact toxicity of Temik, an investigation of the contact action of the compound and its metabolites on plant parasitic nematodes was desired. This work is a study of the *in vitro* effect of Temik, aldicarb sulfoxide and aldicarb sulfone on body activity and stylet movement of second-stage potato cyst nematode larvae *Heterodera rostochiensis* (= *H. rostochiensis* L2) chosen in view of its economic importance as an agricultural pest in Western Europe.

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MATERIALS AND METHODS

H. rostochiensis L2 were hatched from cysts soaked in tomato root diffusate, the larvae for this study were collected 5 days after hatching commenced.

The experimental exposure combined two phases, an initial 24-hr nematicidal treatment and a subsequent 24-hr water treatment. After the hatched larvae were washed in distilled water approximately 20 nematodes were immersed in freshly prepared 1, 5 and 10 ppm solutions of Temik, aldicarb sulfoxide and aldicarb sulfone made up in aerated distilled water. The experimental solutions in flat-bottomed syracuse dishes were kept at 20 C in the dark to prevent photolytic breakdown. Hourly observations of larval activity were made for 6 hr and finally after 24 hr. The nematodes were then thoroughly washed three times with distilled water and placed in aerated distilled water under the same conditions as before and observations made at 1, 3 and 24 hr. The tests were replicated six times.

The experimental observations consisted of counting the body undulations or wave frequency of 10 nematodes in 30 seconds, one undulation taken to represent one complete cycle of movement at the anterior end (10). The results indicated a wide variation in the amplitude of the undulations between both batches and treatments. Thus, to obtain a direct comparison, the percentage of nematodes actively undulating during each observation interval was calculated (16, 5) from the original observations. Nematodes in physical contact with one another were not recorded, thus more than 10 nematodes were used in each sample replicate to compensate for larvae touching and still allow 10 individuals to be observed.

The percentage of larvae exhibiting abnormal stylet movement was determined from the original observations in a similar manner during each observation interval. The stylet movement was defined as axial extrusion and retraction of the stylet.

A separate series of nematicidal contact tests were designed to establish an ED_{50} for the experimental compounds against *H. rostochiensis* L2. According to Trevan (15) an ED_{50} is the median effective dose which will produce a response other than death in half the population. The larvae were treated at various dosage levels for 24 hr, washed and allowed to migrate through a Coldstreem® milk filter (Boots Ltd., Nottingham, U.K.) on a 90-μ nylon sieve immersed in water. Larvae able to migrate through the filter and sieve were collected after 24 hr and counted.

RESULTS

Temik was the most effective toxicant, rapidly inhibiting body activity at the higher dosage level (Fig. 1). After the initial 24-hr nematicide treatment all the larvae treated with 10 ppm Temik were inactive, compared to 86% with the sulfoxide and 65% with the sulfone. The majority of the larvae treated at the 10-ppm dosage recovered some of their former vigor during the subsequent water treatment, 67% of those with aldicarb sulfoxide and 62% with aldicarb sulfone. Effective recovery of larvae treated with 10 ppm of the primary toxicant did not occur, only 8% were able to undulate after 24 hr, and their vigor was much reduced.

The percentage of larvae exhibiting abnormal stylet movement was greatest during exposure to the nematicides, and very little during the water treatment (Fig. 2). The response of the larvae to the high dose was marked after 6 hr, 69% with Temik compared to 15% with the sulfoxide and 5% with the sulfone. After 24 hr Temik 60%, the sulfoxide 30%, while no activity with the sul-

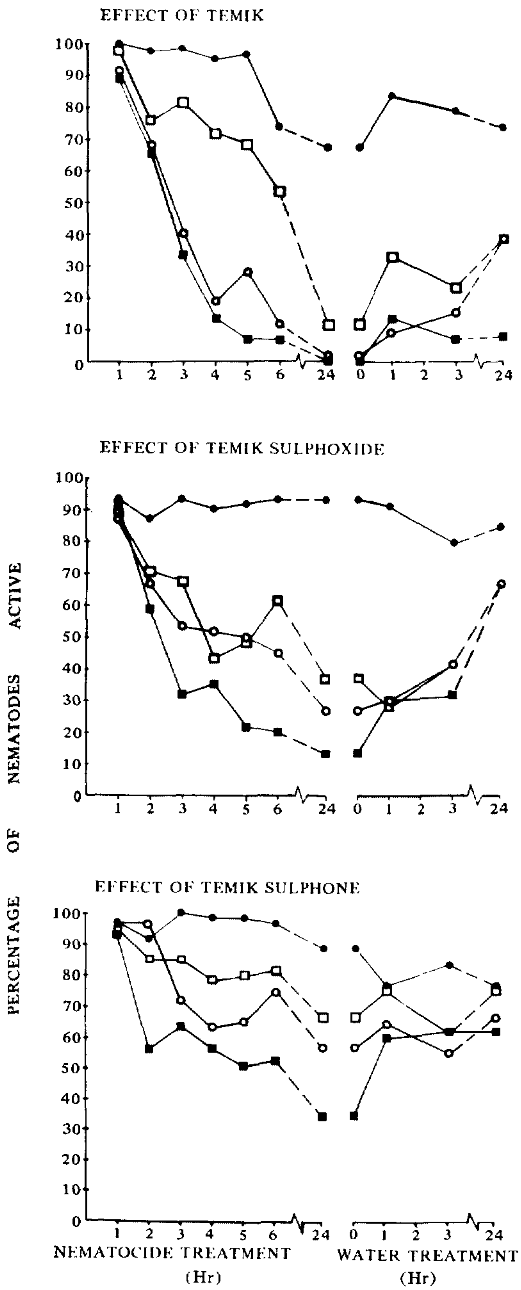

FIG. 1. Percentage of *H. rostochiensis* L2 that exhibited undulatory body movements when treated with different carbamoyloximes at 1, 5 and 10 ppm for 24 hr, followed by a 24-hr aerated water treatment. Key: ●-●-● Control, □-□-□ 1 ppm treatment, ○-○-○ 5 ppm treatment, ■-■-■ 10 ppm treatment.

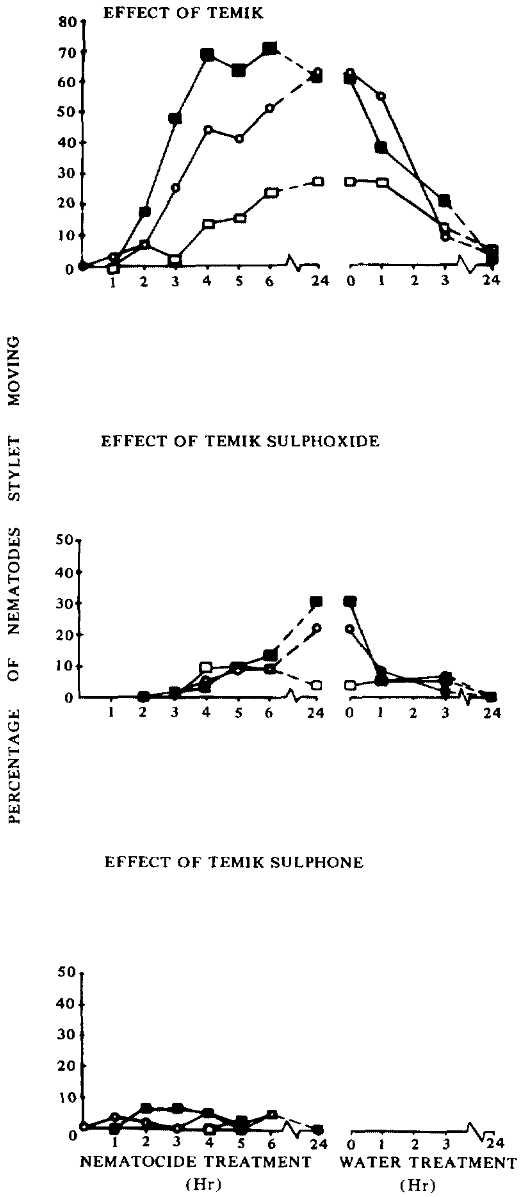

FIG. 2. Percentage of *H. rostochiensis* L2 that exhibited abnormal stylet movement when treated with different carbamoyloximes at 1, 5, and 10 ppm for 24 hr, followed by a 24-hr aerated water treatment. Key: □-□-□ 1 ppm treatment, ○-○-○ 5 ppm treatment, ■-■-■ 10 ppm treatment.

fone was observed. During the second phase (water treatment) the stylet thrusting activity of the treated larvae rapidly ceased.

An ED_{50} of 4.62 ppm with Temik was calculated from the results of the water contact tests, but with concentrations up to 175 ppm with aldicarb sulfoxide and aldicarb sulfone did not produce this level of response.

Discussion

The inhibitory effect of Temik, aldicarb sulfoxide and aldicarb sulfone on the body activity of *H. rostochiensis* L2 is generally reversible, but at higher concentrations with Temik the effect is permanent at least for the experimental duration. The larvae that were unable to recover were not killed but paralyzed, and this condition may last until all the food reserves were utilized and death followed. The carbamoyloximes stimulate stylet activity in larvae which normally exhibit a high rate of activity during hatching (6), but not when simply immersed in water. As the rate of body activity diminishes so the rate of stylet activity increases when the larvae are treated with the primary toxicant and the sulfoxide. The rate of stylet movement slows towards the end of the nematicide treatment with the Temik-treated larvae, the stylet remains extruded between the lips for periods of time longer than the observation intervals.

The method of calculating an ED_{50} is dependent on the experimental design. Whether the response to the nematicide is measured immediately after exposure to the compounds or after the larvae have had sufficient time to recover in water, will affect the number of larvae that are able to move through a filter to the collection vessel. Data on *Panagrellus redivivus* exposed to Temik (13) would suggest that *H. rostochiensis* L2 are more susceptible to lower concentrations of the pesticide.

The inhibition of body activity may be a reflection of anticholinesterase activity, the irreversible nature with Temik at higher concentrations may be due to its continued binding or interaction with the cholinesterase enzymes of the larvae. Penetration of the cuticle may be dependent on the lipophilic or hydrophilic characteristics of the carbamoyloximes (17). When applied topically, Temik which is lipophilic rapidly penetrates the housefly cuticle, while the hydrophilic aldicarb sulfoxide and aldicarb sulfone penetrate more slowly. Detoxication of carbamates has been demonstrated with insects (7), and it may be an association of this factor with the other physical and chemical properties that effects the rate of toxicity and recovery.

Temik is effective as a contact nematicide but in soil where degradation is rapid, aldicarb sulfoxide would remain the agent of control (3). In early spring when the host plants stimulate hatching of the larvae from cysts, the soils would be well saturated and a high water table would be expected. The effect of the water table may neutralize the contact toxic effect of the aldicarb sulfoxide in the soil and thus allow recovery of the hatched larvae. It would also be pertinent to discover whether larvae which have recovered from the effects of the nematicide would still be able to invade plant roots or respond to sex attractants.

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