

## Nematostatic Activity of Oxamyl and N,N-Dimethyl-1-cyanoformamide (DMCF) on *Meloidogyne incognita* Juveniles<sup>1</sup>

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**Abstract:** The nematostatic activity of oxamyl, methyl-N',N'-dimethyl-N-hydroxy-1-thiooxamimidate (oxamyl-oxime) and N,N-dimethyl-1-cyanoformamide (DMCF) was studied by immersing 10 *Meloidogyne incognita* second-stage juveniles into aqueous solutions of various concentrations of each chemical. At concentrations of 500 to 8,000 µg/ml, oxamyl quickly immobilized immersed juveniles. In all other concentrations studied (down to 4 µg/ml), oxamyl stopped or reduced movement of juveniles within 24 hours. DMCF also quickly immobilized juveniles at concentrations of 4,000 and 8,000 µg/ml and reduced movement at 2,000 µg/ml. Lower concentrations had no observed effect on movement. In solutions of the oxime from 2,000 to 8,000 µg/ml, some reduction of movement was observed, but most juveniles maintained some motion over a period of 24 hours. Juveniles were transferred to water from 4,000 µg/ml solutions of oxamyl and DMCF after various intervals of time in order to determine the effect of duration of exposure to the chemicals on the ability of the immobilized juveniles to recover normal motion. Some recovery was observed even after 24 hours of exposure to DMCF, but none after exposure to oxamyl for longer than 40 minutes.

**Key words:** biological activity, *Meloidogyne incognita* (Southern root-knot nematode), metabolites, mode of action, nematostatic, N,N-dimethyl-1-cyanoformamide (DMCF), oxamyl (Vydate), oxamyl-oxime.

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Oxamyl has been known for several years to be effective in controlling nematode invasion of roots (8), but its mode of action has not been clearly elucidated. The fact

that oxamyl applied to foliage is effective in protecting the roots has led to the conclusion that oxamyl itself, or some biologically active metabolite, is translocated to the roots of foliar-treated plants (1,7,8,11). However, the quantities of oxamyl actually found in the root have been extremely small in most studies in which analysis for oxamyl was performed (2,5,11). This supports the view that it may be a metabolite of oxamyl which protects the roots, though to date

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no metabolite of oxamyl has been demonstrated to be biologically active.

While analyzing corn seeds which had been treated with oxamyl as part of our seed-treatment study, two major metabolites of oxamyl were found. These were methyl-N',N'-dimethyl-N-hydroxy-1-thio-oxamimidate (oxamyl's corresponding oxime) and N,N-dimethyl-1-cyanoforamidate (DMCF). The finding of the oxime has been reported in several studies (2,5,11), and DMCF was found in oxamyl-treated fruit (5). The oxime has been reported to lack biological activity (3,11), but the activity of DMCF has not been investigated.

This paper reports the results of preliminary studies on the nematostatic activity of DMCF in relation to that of oxamyl and its oxime.

#### MATERIALS AND METHODS

Analytical standards of oxamyl and the oxime were obtained from E. I. du Pont de Nemours and Co. (Wilmington, Del.). DMCF was prepared using a modification of the method of Harvey and Han (6). Equimolar amounts of sodium cyanide (BDH Chemicals, Toronto, Canada) and dimethylcarbamoyl chloride (Aldrich Chemical Co., Milwaukee, Wis.) were combined in freshly dried (over activated  $K_2CO_3$ ) and distilled acetone and allowed to react at room temperature with stirring for 3 days in a tightly capped Erlenmeyer flask. The acetone was then evaporated and water added to hydrolyze any unreacted dimethylcarbamoyl chloride. DMCF thus formed was extracted with diethyl ether and, after evaporation of the ether, was obtained in good yield (about 70%). Comparison of the mass spectrum of the product with that of a sample of DMCF obtained from Du Pont indicated that the product was in fact DMCF.

The nematodes used in the study were *Meloidogyne incognita* Chitwood second-stage juveniles reared on tomato (*Lycopersicon esculentum* L. cv. Stakeless) and hatched from hand-picked egg masses within 12 hours before use.

The effect of various concentrations of DMCF, the oxime, and oxamyl was studied by placing 10 hand-picked juveniles into 0.1 ml of an aqueous solution of the chemical in a microscope cavity slide. The

juveniles were observed under the microscope for any change in their normal sine-wave pattern of motion. Oxamyl was studied in this way at concentrations of 4, 8, 16, 31, 63, 125, 250, 500, 1,000, 2,000, 4,000, and 8,000  $\mu\text{g/ml}$ , DMCF at 500, 1,000, 2,000, 4,000, and 8,000  $\mu\text{g/ml}$ , and the oxime at 2,000, 4,000, and 8,000  $\mu\text{g/ml}$ . Ten juveniles were also placed in 0.1 ml of distilled water as a control. The experiments with oxamyl from 4  $\mu\text{g/ml}$  to 31  $\mu\text{g/ml}$  were performed in duplicate and those from 63 to 500  $\mu\text{g/ml}$  in triplicate. The remaining tests were not replicated. It had previously been shown that protection of alfalfa seedlings grown from oxamyl-treated seed was not substantially improved by increasing the oxamyl concentration above 8,000  $\mu\text{g/ml}$  (10). Therefore, concentrations higher than this were not studied in this experiment.

The effect of duration of exposure to solutions of oxamyl and DMCF on the juveniles' ability to recover normal motion was studied using 4,000  $\mu\text{g/ml}$  solutions because juveniles were quickly immobilized at this concentration of each chemical. At 10-minute intervals, 10 juveniles were transferred from the 4,000  $\mu\text{g/ml}$  oxamyl solution to distilled water and observed for recovery of normal motion. Juveniles were transferred to water from the 4,000  $\mu\text{g/ml}$  DMCF solution after 4, 8, 12, and 24 hours and similarly observed.

In all the experiments, evaporation of the aqueous solutions was minimized by using humidity chambers consisting of covered petri dishes containing a layer of water and supports for the microscope slides. All experiments were conducted at ambient room temperature (approximately 23 C).

#### RESULTS

In 8,000  $\mu\text{g/ml}$  solutions of oxamyl and DMCF, *M. incognita* juveniles were immobilized in random configurations within 10 minutes. After 6 hours in 8,000  $\mu\text{g/ml}$  oxamyl, the juveniles were observed to be straight and motionless, the position normally assumed upon death. Juveniles in 8,000  $\mu\text{g/ml}$  DMCF were observed to be coiled after 2 hours and straight and motionless after 21 hours. Juveniles maintained slow but strong movement for at least 7 hours in an 8,000  $\mu\text{g/ml}$  solution

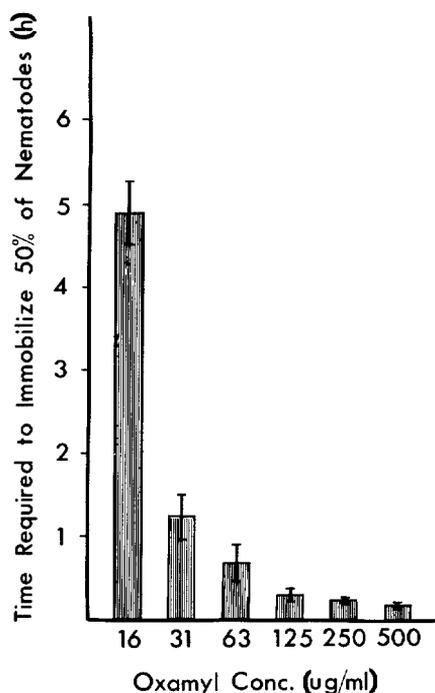


FIG. 1. Approximate time required to immobilize 50% of the nematodes immersed in six concentrations of oxamyl. Bars indicate  $\pm$  one standard deviation.

of the oxime, and when observed after 24 hours, six still showed some degree of movement. The juveniles placed in distilled water exhibited normal movement over a 24-hour period.

In solutions of oxamyl from 500 to 4,000  $\mu\text{g}/\text{ml}$ , the motion of the juveniles was quickly reduced and they exhibited only some twitching of the tail or spasmodic flexing about midpoint in the body. After several minutes, motion was completely stopped and the juveniles were in random configurations. When observed after 20 hours all juveniles were straight and motionless.

As the concentration of oxamyl decreased below 500  $\mu\text{g}/\text{ml}$ , the juveniles maintained motion for longer periods of time. Coiling or abnormal angular bending, as described by Wright et al. (11), was common. Figure 1 illustrates the trend observed in terms of the approximate time required to immobilize 50% of the juveniles versus the concentration of oxamyl. In concentrations above 31  $\mu\text{g}/\text{ml}$ , all nematodes stopped moving within 2.5

TABLE 1. Effect of duration of exposure to 4,000  $\mu\text{g}/\text{ml}$  solutions of oxamyl and DMCF on recovery of *M. incognita* juveniles after transfer to distilled water.

Chemical	Duration of exposure	Maximum observed recovery	
		%*	Time of observation (hours)
Oxamyl	10 minutes	100	24†
	20 minutes	70	24†
	30 minutes	90	30†
	40 minutes	30	28†
DMCF	4 hours	100	0.5‡
	8 hours	60	24‡
	12 hours	30	12‡
	24 hours	30	3§

\* Percentage of juveniles which recovered some degree of motion.

† Total period of observation = 30 hours.

‡ Total period of observation = 24 hours.

§ Total period of observation = 12 hours.

hours. In the 4 and 8  $\mu\text{g}/\text{ml}$  oxamyl solutions, movement of the juveniles was considerably slowed after 21 hours, but most were still moving. In all concentrations below 500  $\mu\text{g}/\text{ml}$  almost all juveniles remained in random configurations after 20 hours in solution.

Nematodes immersed in 4,000  $\mu\text{g}/\text{ml}$  DMCF were immobilized in random configurations within 10 minutes. Some straightening and coiling were observed in the first 2 hours. In 2,000  $\mu\text{g}/\text{ml}$  DMCF, the motion of the juveniles was slowed but not stopped and all juveniles were still moving after 18.5 hours. No effect on motion resulted from immersion in 500 or 1,000  $\mu\text{g}/\text{ml}$  DMCF for up to 18.5 hours.

Nematodes immersed in a 4,000  $\mu\text{g}/\text{ml}$  solution of the oxime showed slow normal movement for at least 7 hours and the majority still showed slow movement after 24 hours. Most of the juveniles immersed in a 2,000  $\mu\text{g}/\text{ml}$  solution of the oxime continued normal movement over a 24-hour period.

The ability of immobilized juveniles to recover normal motion after transfer to water from 4,000  $\mu\text{g}/\text{ml}$  solutions of oxamyl and DMCF is summarized in Table 1. Recovery from exposure to 4,000  $\mu\text{g}/\text{ml}$  oxamyl was slow, and juveniles exposed for longer than 40 minutes did not recover any movement after transfer to water. On the

other hand, complete recovery of nematodes exposed to 4,000  $\mu\text{g}/\text{ml}$  DMCF for 4 hours was observed within 30 minutes of transfer back to water, and some recovery of movement was observed after immersion in the DMCF solution for as long as 24 hours.

#### DISCUSSION

The results indicate that DMCF exhibits a degree of biological activity, though not to the same extent as that of oxamyl. The activity of the oxime appears to be very weak relative to DMCF and oxamyl, as demonstrated by the continued motion exhibited by juveniles over a 24-hour period in all oxime concentrations studied.

Oxamyl in high concentration appears to be lethal to nematodes exposed to the chemical for an extended period of time. This is evidenced by the irreversible straightening of nematodes exposed to 4,000  $\mu\text{g}/\text{ml}$  oxamyl for longer than 40 minutes. However, recovery of motion by nematodes after short exposures to 4,000  $\mu\text{g}/\text{ml}$  oxamyl was observed, indicating a reversible nematostatic effect during the period of exposure. Oxamyl in low concentration appears to act not as a nematocide but as a nematostat, as evidenced by the lack of straightening of nematodes in oxamyl solutions below 500  $\mu\text{g}/\text{ml}$ . Also, Wright et al. (11) found that *M. incognita* second-stage juveniles recovered normal activity upon transfer to water after 24-hour exposure to a 32  $\mu\text{g}/\text{ml}$  oxamyl solution.

DMCF evidenced a reversible nematostatic effect at a concentration of 4,000  $\mu\text{g}/\text{ml}$ , allowing some recovery of nematodes even after 24-hour exposure to the chemical. The nematocidal effect of DMCF at 4,000  $\mu\text{g}/\text{ml}$  was much weaker than that of oxamyl at the same concentration. Since DMCF had no observed effect on the activity of the juveniles at concentrations below 2,000  $\mu\text{g}/\text{ml}$ , it is unlikely that it would have any nematostatic effect at the concentrations to be expected in roots as a result of foliar application of oxamyl.

It has been proposed that oxamyl itself, even when present in very low concentrations as a result of translocation from treated foliage (11), protects roots by inhibiting orientation or feeding ability of nematodes

(4,9,11). The possibility that the active agent is not oxamyl itself, but a metabolite of oxamyl, has been acknowledged (7,9), but biological activity of any oxamyl metabolites has not been demonstrated to date.

DMCF is the first metabolite of oxamyl shown to possess a degree of biological activity against nematodes. While this study found the activity of DMCF to be far lower than that of oxamyl with regard to nematostasis, DMCF may have some other type of activity. The reason for its nematostatic activity is not clear, since the DMCF molecule does not contain a carbamate group and thus probably does not inhibit acetylcholinesterase. Further investigations are planned to determine whether DMCF acts by some other mechanism in low concentrations, such as inhibition of nematode orientation or feeding, and whether there are other metabolites of oxamyl which possess biological activity against nematodes.

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