

Mode-of-action of the carbamate nematicides cloethocarb, aldicarb and carbofuran on *Heterodera schachtii*.

1. Contact activity

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SUMMARY

The non-systemic contact mode-of-action of the systemic carbamate nematicides cloethocarb (Lance®), aldicarb (Temik®) and carbofuran (Curaterr®) on *Heterodera schachtii* was studied in the laboratory. The nematicides suppressed hatch at concentrations higher than would occur under practical field conditions. Juvenile mobility was, in general, inhibited at concentrations from 5-10 ppm. Carbofuran also inhibited juvenile mobility at 1 ppm and aldicarb at 0.1 ppm. Orientation and penetration into roots was not affected by carbamate concentrations of 0.0001-0.1 ppm. The results suggest that reduction of juvenile mobility is the main factor responsible for carbamate activity against *H. schachtii*. Cloethocarb showed the same activity mechanisms as aldicarb and carbofuran.

RÉSUMÉ

Mode d'action sur Heterodera schachtii de nématicides à base de carbamates : cloéthocarbé, aldicarbé et carbofuran. 1. Action de contact

Le mode d'action sur *Heterodera schachtii* de nématicides de contact non systémiques à base de carbamates — cloéthocarbé (Lance®), aldicarbé (Temik®) et carbofuran (Curaterre®) — a été étudié au laboratoire. Ces nématicides inhibent l'éclosion à des concentrations plus élevées que celles existant dans les conditions du champ. La mobilité des juvéniles est, en général, inhibée à des concentrations de 5 à 10 ppm. Le carbofuran inhibe cette mobilité des juvéniles à un taux de 1 ppm et l'aldicarbé à un taux de 0,1 ppm. L'orientation des juvéniles et leur pénétration dans les racines ne sont pas modifiées par des concentrations de 0,0001 à 0,1 ppm. Ces résultats suggèrent que le principal facteur responsable de l'action des carbamates contre *H. schachtii* est la diminution de la mobilité des juvéniles. Le cloéthocarbé fait montre des mêmes mécanismes d'activité que l'aldicarbé et le furadan.

Carbamate insecticides/nematicides are commonly used plant protection components in sugarbeet production worldwide. They have been shown to be effective in reducing early root infection of sugarbeet seedlings by *Heterodera schachtii* usually resulting in significant increases in yield.

A new active ingredient cloethocarb has been shown to be active against nematodes (Harries *et al.*, 1980) and has a lower mammalian toxicity level than other carbamate nematicides. Nothing is known about its mode-of-action. In this study a comparative analysis was made of the contact mode-of-action of cloethocarb (Lance®), aldicarb (Temik®) and carbofuran (Curaterr®) towards *H. schachtii* on sugarbeet.

Materials and methods

Carbamate solutions were produced by grinding the compounds, which are highly water soluble, in mortars and dissolving the powder in tap water on a stirrer for 12 h at 22 °C. The solubility of these compounds in water is : carbofuran - 700 mg/l, aldicarb - 6000 mg/l, cloethocarb - 700 mg/l. The non-water soluble granulate carriers were then removed by filtration.

HATCH

Dilution series of the primary carbamate solutions were prepared using tap water. The final concentration with aldicarb was 0.00006 ppm, with carbofuran

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0.007 ppm and cloethocarb 0.017 ppm. The control was prepared with water. 7.5 ml of each dilution was placed in a test tube and 1000 *H. schachtii* eggs were added to each test tube. In order to prevent evaporation, the test tubes were covered with aluminium foil and incubated in darkness at $23 \pm 2^\circ\text{C}$.

The eggs and J2 in 1 ml were counted 7 days after inoculation. The ratio of eggs with unhatched J2 to hatched J2 was determined. The sum of the egg and juvenile counts was at least 100 for each counting. The number of eggs with underdeveloped contents was not significant to justify their determination in counts.

The effects of the carbamates on hatch rate was determined using the formula of Clarke and Shepherd (1964) :

$$\text{hatch rate} = \frac{\text{hatch \% (carbamate)}}{\text{hatch \% (control)}} \times 100$$

JUVENILE MOBILITY

The effects of the carbamates on J2 mobility were determined using the "direct photography" method (Kunz & Klingler, 1976). *H. schachtii* J2 in water were added to solutions of cloethocarb, aldicarb and carbofuran, so that 10, 5, 1 and 0.1 ppm solutions were produced that had a nematode density of 100 J2/ml. Tap water was used in the control.

At fixed intervals, from 0.5 to 100 h after the beginning of the test, a 10 ml nematode suspension was taken from each carbamate solution and immediately adjusted to a density of ca 1 J2 per μl ; 20 μl was then pipetted into the center of a Petri dish containing 3% water agar. The surface water on the dishes was evaporated prior to inoculation under a laminar flow hood. The actual number of juveniles transferred was determined under the microscope.

After the 20 μl drop had evaporated (ca. 10 min.) the J2 moved randomly on the surface of the agar, leaving behind typical tracks (Rode & Staar, 1961; Rode, 1970). The track patterns in the Petri dishes were recorded at periods from 0.5 to 100 h using « direct photography ». The negative was enlarged 10 times with the aid of a slide projector onto the sensor plate of a "MOB Kontron, AM 3" planimeter (Fig. 1). The length of the tracks was determined by tracing them with the planimeter cursor pen. The average distance travelled per juvenile was calculated based on the length of all tracks in the replicate dishes. Juvenile mobility for each time period was studied using five replicates and its mean was calculated in percent mobility relative to control.

ORIENTATION AND PENETRATION

The tests were performed using a modified "sand-block method" (Kerstan & Röpke, 1977). A layer of chemically pure sand (grain size 0.1-0.3 mm) in a block shape of 5 cm length, 2.5 cm width and 0.5 cm height

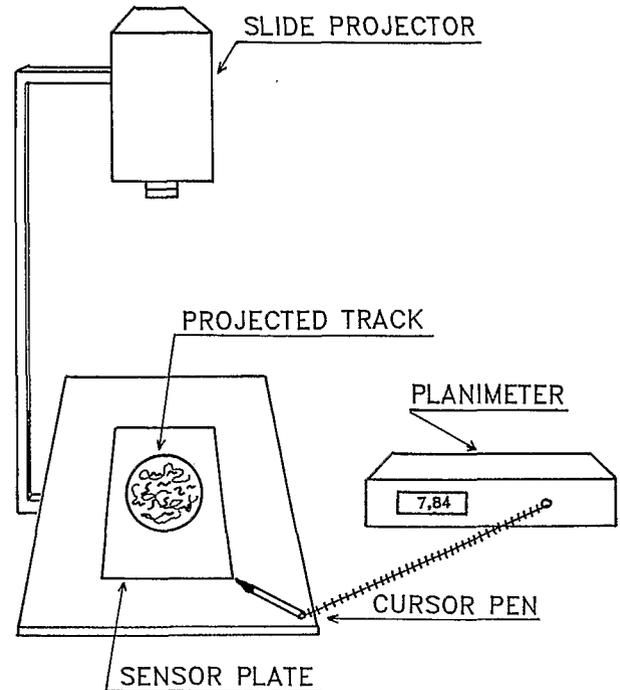


Fig. 1. Apparatus with slide projector used to project black and white negatives of nematode tracks produced on an agar plate onto a planimeter for measurement of nematode mobility under the influence of nematicides.

was placed in Petri dishes (14 cm diameter). Reference lines on the base of the Petri dishes were used to mark off five sand-block sectors (Fig. 2).

A single sugarbeet plant, in the emerging second leaf pair stage, was inserted in sector 5 and 2000 *H. schachtii* juveniles were pipetted onto sector 1. The entire sand-block was saturated with solutions of cloethocarb, aldicarb or carbofuran at concentrations of 0.1, 0.01, 0.001 or 0.0001 ppm. The sand-blocks in the control were

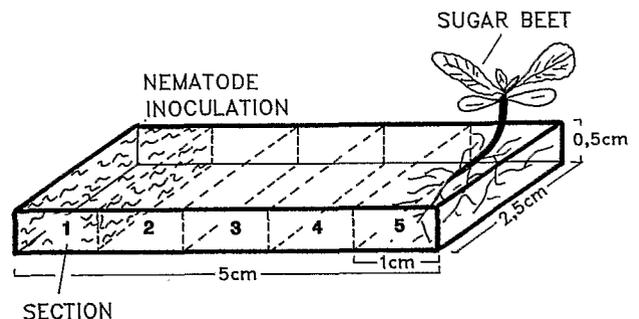


Fig. 2. Sand-block orientation chamber for testing the orientation and penetration of *Heterodera schachtii* juveniles.

saturated with water. An additional control was prepared without sugarbeets. The Petri dishes were sealed in order to protect them against evaporation.

After 6 days at 20 ± 1 °C in a climatically controlled chamber, the sand of each sector was placed individually in a test tube, mixed with 10 ml tap water and shaken vigorously. After the sand fraction had settled out, the nematodes were counted. In addition to the number of juveniles in each sector, the number of those penetrated into the roots was determined by root staining.

Results

HATCH

The saturated solution of cloethocarb (1700 ppm) suppressed hatch almost completely (Fig. 3). The suppressive effect decreased with decreasing concentration of the active ingredient. Carbofuran did not reduce hatch as effectively even in the saturated solution. Carbofuran suppressed hatch significantly when compared to the control down to a concentration of 70 ppm. Aldicarb strongly suppressed hatch at concentrations between 60 and 6000 ppm and even 6 ppm suppressed

Table 1

Distribution (%) of *Heterodera schachtii* J2 in sectors of the sand-block orientation chamber (n = 6).

Treatment	Sector					χ^2
	1	2	3	4	5	
CONTROL no plant	48.3	17.2	8.4	10.1	15.5	a
CONTROL with plant	11.7	2.1	0.6	0.9	84.6	b
CLOETHOCARB						
0.1 ppm	9.8	1.2	1.1	0.6	87.3	b
0.01 ppm	16.8	6.3	3.2	2.0	71.9	b
0.001 ppm	17.5	2.1	1.2	0.7	78.4	b
0.0001 ppm	18.5	1.7	0.5	1.1	78.9	b
ALDICARB						
0.1 ppm	9.9	1.7	0.7	0.6	87.0	b
0.01 ppm	16.9	1.6	1.0	0.9	79.5	b
0.001 ppm	10.6	1.5	0.8	0.7	86.4	b
0.0001 ppm	15.2	2.0	2.2	1.9	78.6	b
CARBOFURAN						
0:1 ppm	11.5	2.7	1.2	0.8	83.7	b
0.01 ppm	11.6	2.3	1.0	1.3	83.8	b
0.001 ppm	14.7	2.6	1.0	1.3	80.3	b
0.0001 ppm	16.8	4.5	1.4	1.5	75.7	b

Distributions with the same letters are not significantly different following the χ^2 -test.

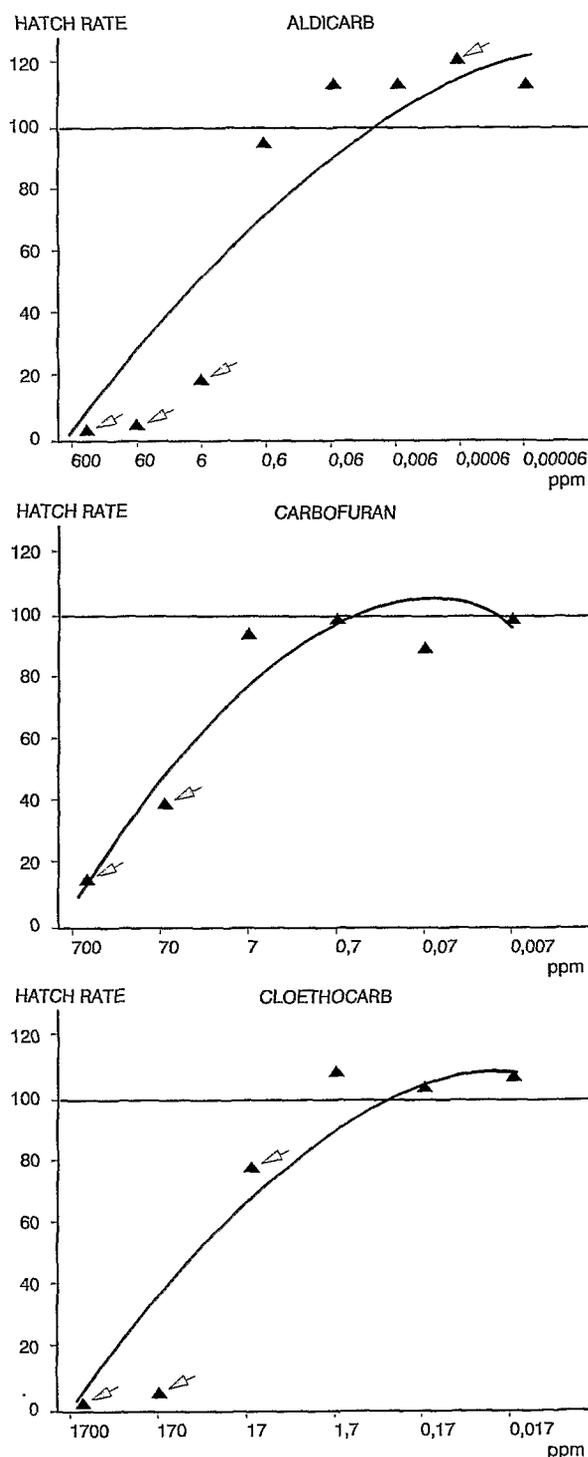


Fig. 3. Effect of aldicarb (top), carbofuran (center) and cloethocarb (bottom) on the hatch rate of *Heterodera schachtii* as a function of carbamate concentration : function curves calculated from the data points (▲); ← = significantly different from the control (100 %) (mean of ten repetitions).

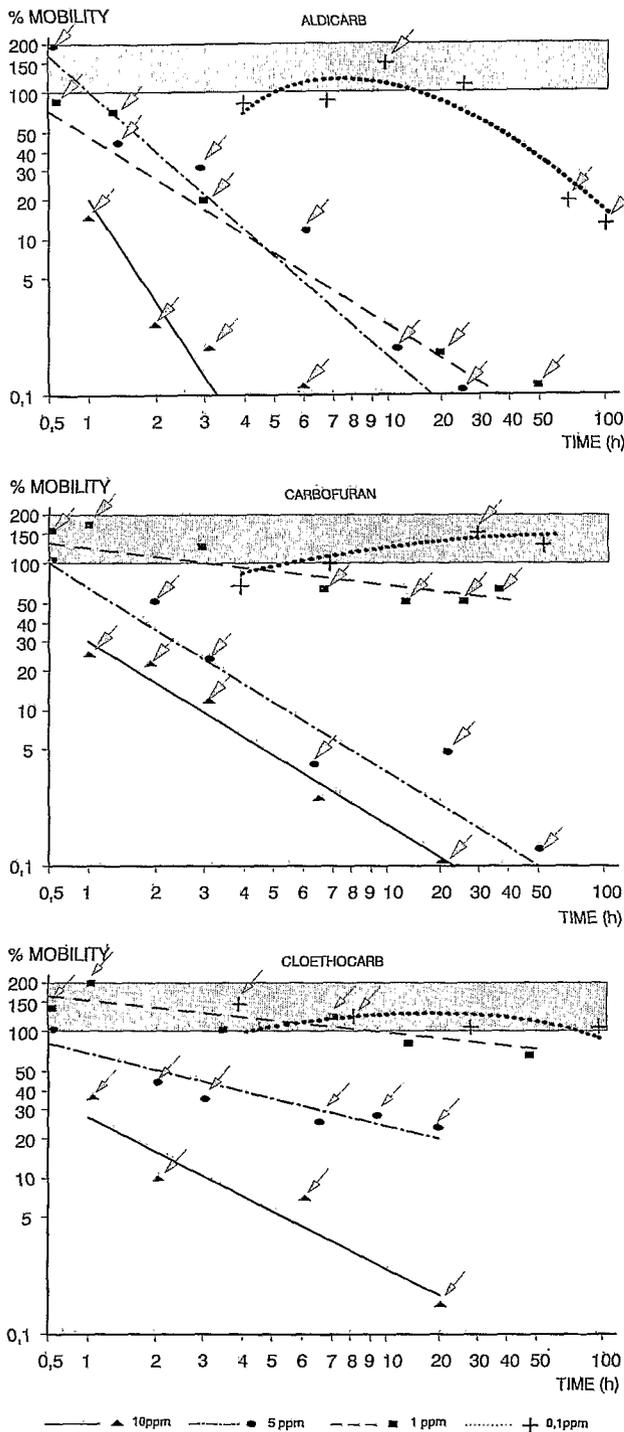


Fig. 4. Effect of aldicarb (top), carbofuran (center) and cloethocarb (bottom) on juvenile mobility as a function of time and carbamate concentration; ← = significantly different from the control (100 % mobility) (mean of five repetitions).

hatch. Contrary to the activity curve for cloethocarb and carbofuran, aldicarb at low concentrations stimulated hatching.

Table 2

Heterodera schachtii root penetration rates of J2 in sector 5 of the sand-block orientation chamber under the influence of carbamates (n = 6).

ppm	0.1	0.01	0.001	0.0001	0
Control with plant	—	—	—	—	54.1
Cloethocarb	65.2	47.3	56.8	54.6	—
Aldicarb	86.4	48.8	69.9	82.5	—
Carbofuran	90.5	80.4	75.8	53.1	—

Mean values are not different from one another according to Duncan's multiple range test at $P \leq 0.05$.

JUVENILE MOBILITY

Partial inhibition of juvenile mobility occurred at concentrations of 5 and 10 ppm cloethocarb with nearly total inhibition after 20 h at 10 ppm (Fig. 4). Treatment levels of 1 and 0.1 ppm stimulated mobility. At a concentration of 1 ppm, mobility increased significantly 147 %, 209 % and 123 % after 0.5, 1 and 6.5 h, respectively. At 0.1 ppm a significant stimulatory effect took place after 4 and 8 h (123 % and 111 %). A stable inhibitory effect on mobility was not observed at the lower concentrations.

Immobilization of the J2 by aldicarb occurred at a concentration of 10, 5 and 1 ppm after 6, 25 and 50 h, respectively (Fig. 4). Mobility was reduced 87 % at a concentration of 0.1 ppm compared to the control after 100 h. As with cloethocarb, aldicarb also stimulated juvenile mobility. After 0.5 h at 5 ppm and after 9 h at 0.1 ppm, an increase in mobility was measured. This increase was 79 % and 29 %, respectively, over that of the control.

10 and 5 ppm carbofuran inhibited J2 mobility after 22 and 51 h, respectively (Fig. 4). At a concentration of 1 ppm, carbofuran had a mobility-inhibiting effect which reached a maximum of 28.7 % lower than the control value. As with cloethocarb and aldicarb, mobility increases were also observed with carbofuran. At concentrations of 1 ppm, carbofuran stimulated mobility after 0.5 h by 23 %, after 1 h by 48 % and after 3 h by 33 % compared to the control. Mobility was increased by 27 % at a concentration of 0.1 ppm and a time of 31 h.

ORIENTATION AND PENETRATION

In the absence of a host plant, 48.3 % of the untreated juveniles remained in sector 1 and a significantly lower percentage of juveniles was found in each of sectors 2-5 (Table 1). When a sugarbeet was present, 84.6 % of all untreated J2 migrated into sector 5.

The application of cloethocarb, aldicarb and carbofuran did not significantly affect the distribution of the juveniles in the sectors compared with the distribution achieved for the untreated juveniles. Thus, carbamates at concentrations from 0.0001-0.1 ppm did not adversely affect J2 orientation.

There were no significant differences between treatments in the invasion rates of those juveniles that had reached sector 5 (Table 2). Neither the type of carbamate nor the concentration of the active ingredient had any effect on penetration of juveniles into the root system.

Discussion

Water soluble carbamate insecticides/nematicides can influence both juvenile activity as well as unhatched juveniles in encysted eggs. Hatch rate determines the maximum number of infective juveniles that can penetrate a host. An alteration in hatch behaviour induced by the action of a carbamate compound, therefore, directly affects the intensity of infection. For this reason, the determination of the *in vitro* hatch behaviour is an important criterion in estimating the effectiveness of a carbamate nematicide (Kämpfe, 1971).

In our investigations, cloethocarb, aldicarb and carbofuran inhibited *H. schachtii* hatch. The intensity of inhibition was correlated with concentration. Aldicarb inhibited hatch at very low concentrations when compared to cloethocarb and carbofuran. Similar levels of hatch inhibition have been detected at concentrations of 5 ppm (Steudel, 1972; Hough & Thomason, 1975; Steele & Hodges, 1975; Steele, 1977). Nevertheless, hatch inhibition detected here is of minor importance in nematode control due to the nematicide concentration normally attained in the field.

Juvenile mobility was studied on water agar using orientation tracks produced on the agar surface (Rode & Staar, 1961; Sandstedt, Sullivan & Schuster, 1961; Kunz & Klingler, 1976) whose length are an exact measure of mobility. All three carbamates had a negative influence on mobility. Similar effects were observed by other workers with carbamates on *H. schachtii* (Hough & Thomason, 1975; Batterby, 1979). This mode-of-action is of practical importance because the concentrations required for inhibition are reached in the field at recommended rates. The fact that a stimulatory effect exists at lower concentrations indicates that the activity of these carbamates occurs in two stages: a stimulatory and an inhibitory phase.

Because of their cholinesterase inhibiting activity, carbamates may disrupt juvenile orientation and host recognition. Disruption in orientation and host recognition, however, are of minimal importance at concentrations where juvenile mobility is inhibited. Therefore, the influence of the carbamates on orientation was tested at concentrations that did not alter mobility. Orientation of the juveniles was not altered by the three carbamates at concentrations of 0.0001-0.1 ppm. Juvenile attraction to the sugarbeet seedlings was not impaired. Furthermore, these concentrations did not affect root penetration. Reductions in *Pratylenchus penetrans* and *Meloidogyne incognita* penetration after treatment with 10-25 ppm carbofuran, however, have been reported (Di Sanzo, 1973).

Our results demonstrate that the *H. schachtii* infection process on sugarbeet is negatively influenced by cloethocarb, aldicarb and carbofuran. The reduction of juvenile mobility is the most important component of the mode-of-action responsible for limiting early root infection.

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