Mode-of-action of the carbamate nematicides cloethocarb, aldicarb and carbofuran on *Heterodera schachtii*. 2. Systemic activity

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SUMMARY

The systemic activity of cloethocarb (Lance®), aldicarb (Temik®) and carbofuran (Curaterr®) against *Heterodera schachtii* in sugarbeet was studied. Cloethocarb caused an incomplete disruption in juvenile orientation to the root and a reduction in penetration. Aldicarb and carbofuran treated sugarbeets did not induce these effects on the nematode. The development and growth rate of juveniles that penetrated the roots was retarded in cloethocarb and aldicarb but not in carbofuran treated plants. The results demonstrate that cloethocarb and aldicarb have a minor degree of curative activity toward *H. schachtii*.

RÉSUMÉ

Mode d'action sur Heterodera schachtii *de nématicides à base de carbamates : cloéthocarbe, aldicarbe et carbofuran. 2. Action systémique.*

L'action systémique du cloéthocarbe (Lance®), de l'aldicarbe (Temik®) et du carbofuran (Curaterre®) envers *Heterodera schachtii* sur betterave à sucre a été étudiée. Le cloéthocarbe inhibe incomplètement l'orientation des juvéniles vers les racines et diminue le taux de pénétration. Les betteraves à sucre traitées par l'aldicarbe et le carbofuran ne provoquent pas cet effet sur le nématode. Le développement et le taux de croissance des juvéniles ayant pénétré dans les racines sont retardés chez les plantes traitées par le cloéthocarbe et l'aldicarbe, mais non dans le cas du carbofuran. Ces résultats démontrent que le cloéthocarbe et l'aldicarbe ont un faible pouvoir curatif envers *H. schachtii*.

Whereas fumigant nematicides cause a high degree of nematode mortality in the soil, carbamates, at concentrations presently used in the field, do not cause direct mortality (Evans, 1973; Nelmes, Trudgill & Corbett, 1973; Wright, 1980). Carbamates inhibit acetyl-cholinesterase at the nerve synapse causing malfunctioning of the muscular and other organic systems in the nematode (Draber, 1970; Wright, 1980, 1981). Disruption of these systems can greatly affect nematode movement and behaviour and ultimately alter the infection process of the parasite, either by delaying or reducing penetration. Carbamate effects on nematodes are reversible. Nematode activity is restored after degradation or dilution of the carbamates in the rhizosphere and the nematode is able to penetrate and damage the root.

In part 1, the activity of cloethocarb, aldicarb and carbofuran on *Heterodera schachtii* hatch, mobility, orientation and penetration was studied (Hartwig & Sikora, 1991). In these investigations we examined the indirect activity of the carbamates or the systemic effects on *H. schachtii* orientation, penetration, development and growth in sugarbeet.

Materials and methods

ORIENTATION AND PENETRATION

The influence of the carbamates on J2 orientation and penetration was studied using the " sand-block method " (Hartwig & Sikora, 1991). Sugarbeet seedlings were treated for 2 weeks in a sand substrate with cloethocarb, aldicarb or carbofuran (all 5 % granular formulations) at an application rate of 1 g/m row. Control plants were grown in untreated flats. Immediately before being inserted into sector 5 of the sand-block, the roots were thoroughly rinsed in running tap water to remove any carbamate residue adhering to the root surface.

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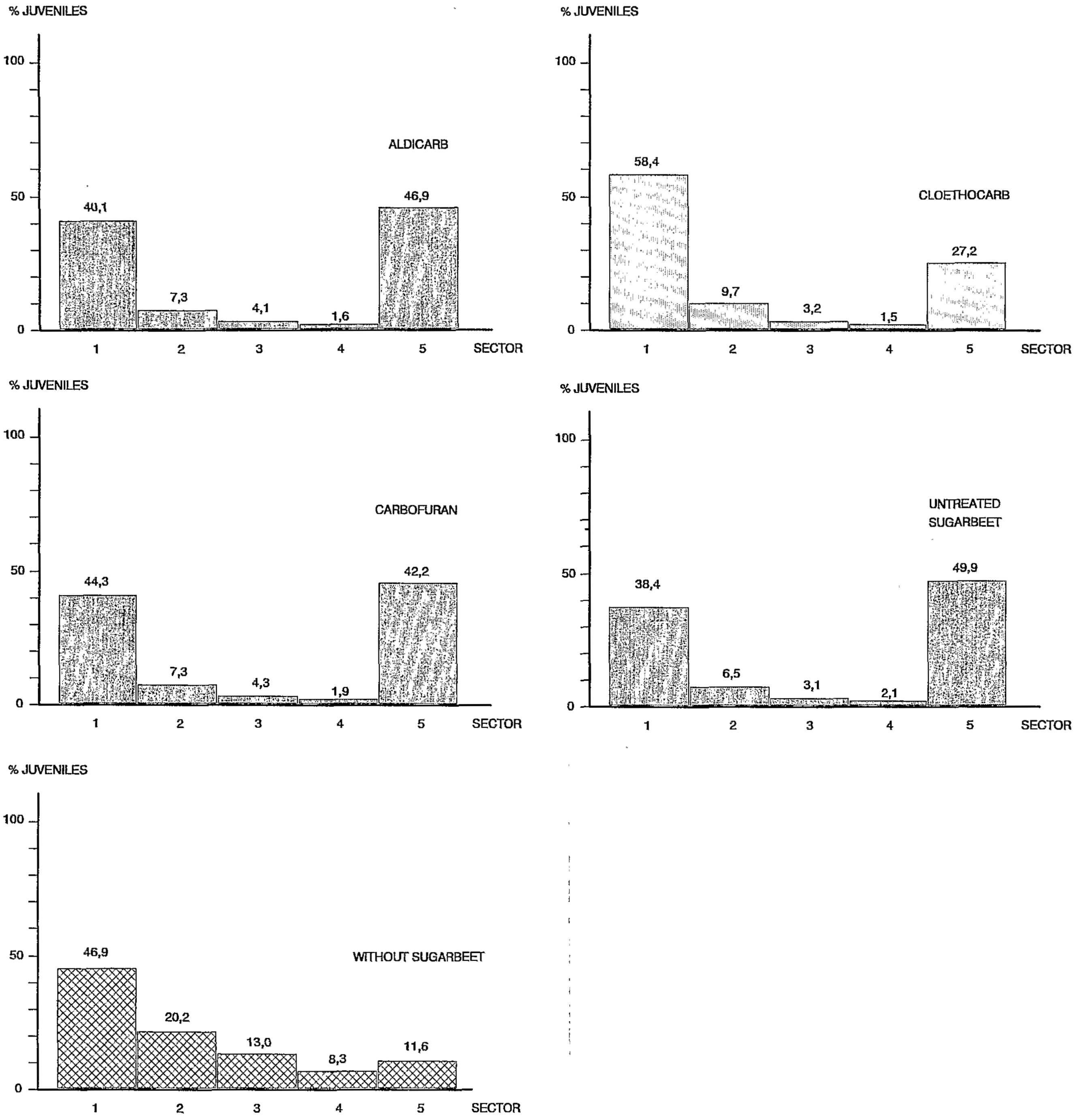

Fig. 1. Distribution of *Heterodera schachtii* J2 in sectors of the sand-block orientation chamber as influenced by sugarbeet treated with carbamates, untreated sugarbeet and without sugarbeet in sector 5. (Distributions significantly different following the χ^2-test are indicated by different bar patterns; mean of ten repetitions).

The sand-block was moistened with tap water and incubated for 48 h to allow a root diffusate gradient to form. Approximately, 1000 *H. schachtii* J2 in 100 µl tap water were then pipetted onto sector 1. The sand-block was saturated with tap water and the Petri dish containing the sand-block sealed. An additional treatment without sugarbeets acted as a control. The experiment was terminated 5 days after nematode inoculation. The distributions of juveniles in the sand-block sectors of the different treatments were compared with one another using a χ^2-test. The percentage of J2 which penetrated the roots in sector 5 based of the total number which reached sector 5 was calculated as the penetration rate.

GROWTH AND DEVELOPMENT

Sugarbeets cv. Kawemono were raised in PVC pots containing 250 ml sand until the emerging second leaf pair was developed. Each pot was then inoculated with 5000 *H. schachtii* juveniles. After 3 days the roots were washed under running tap water to remove any J2 adhering to the root surface.

The sugarbeets were re-potted into PVC pots containing 250 ml steam-sterilized field soil without nematodes. Cloethocarb, aldicarb and carbofuran were added at an application rate of 1 g/m row and the control plants remained untreated.

The plants were placed in a greenhouse at 20 ± 5 °C with 16 h supplemental illumination using sodium vapor lamps. The test was evaluated when the first nematodes in the untreated sugarbeets had reached the 4th juvenile stage. Root systems were then individually stained in acid-fuchsin and macerated with water in an ultra turrax. The resulting mixture was kept in suspension using a vibromixer and a 1 ml sample from the suspension was placed in a Hawksley counting chamber. The number of nematodes in each developmental stage was counted. The length and the maximum width of 25 randomly selected nematodes were measured to determine the volume (Müller, Rehbrock & Wyss, 1981).

Results

ORIENTATION AND PENETRATION

Definite orientation of the juveniles to the untreated sugarbeet seedlings was observed when compared to nematode movement in the treatment lacking sugarbeet (Fig. 1). Whereas 49.9 % of the juveniles were collected in sector 5 of the sand-block containing untreated sugarbeet, only 11.6 % of the J2 accumulated in sector 5 of the sand-block without sugarbeets.

Sugarbeet seedlings treated with cloethocarb had an orientation pattern significantly different from that of the untreated plants (Fig. 1). More J2 remained in sector 1 and fewer nematodes accumulated in sector 5 when compared to the untreated sugarbeet controls. The distribution pattern of the cloethocarb treatment was also significantly different from that in the treatment without sugarbeet. Cloethocarb treated plants also had lower numbers of nematodes in sector 2-4 and high percentages in 1 and 5 than in the control.

Aldicarb and carbofuran treated sugarbeet seedlings had J2 orientation patterns that were not statistically different from the untreated sugarbeets (Fig. 1). The penetration rate in cloethocarb treated sugarbeets was significantly lower than the penetration rate in untreated roots, whereas it was not significantly different from the penetration rates for carbofuran or aldicarb (Table 1). The penetration rates for aldicarb and carbofuran were not significantly different from that of the untreated control. Therefore, cloethocarb treated sugarbeets not only disrupted J2 orientation, but also had an inhibitory effect on penetration. By contrast, treatments with aldicarb and carbofuran did not alter J2 orientation nor inhibit penetration.

Table 1

Heterodera schachtii penetration rates in sand-block orientation chambers as influenced by carbamate treated sugarbeet.

Treatment	*Penetration rate*
Control (untreated sugarbeets)	95.3 a
Cloethocarb treated sugarbeets	80.9 b
Aldicarb treated sugarbeets	90.6 ab
Carbofuran treated sugarbeets	90.4 ab

Mean values followed by the same letter are not significantly different according to Duncan's multiple range test at $P \leq 0.05$, $n = 10$.

GROWTH AND DEVELOPMENT

In the roots of untreated sugarbeet seedlings, 25 nematodes reached the adult stage, 101 reached the J4 stage and 74 remained in the second and third stages (Fig. 2). A similar juvenile ratio was detected in roots treated with carbofuran. Cloethocarb inhibited or delayed J2 development. Compared to the control, a higher percentage of juveniles remained in the J2 and J3 stages, while the percentage of J4 and adults was lower. The strongest inhibition in nematode development was observed with aldicarb.

Depending on the treatments used, the volume classes have the same significances in the χ^2-test as the distribution of developmental stages. Thus, the distribution of the carbofuran treatment corresponds to the control distribution (Fig. 3). On the other hand, the cloethocarb distribution has a higher percentage of nematodes in

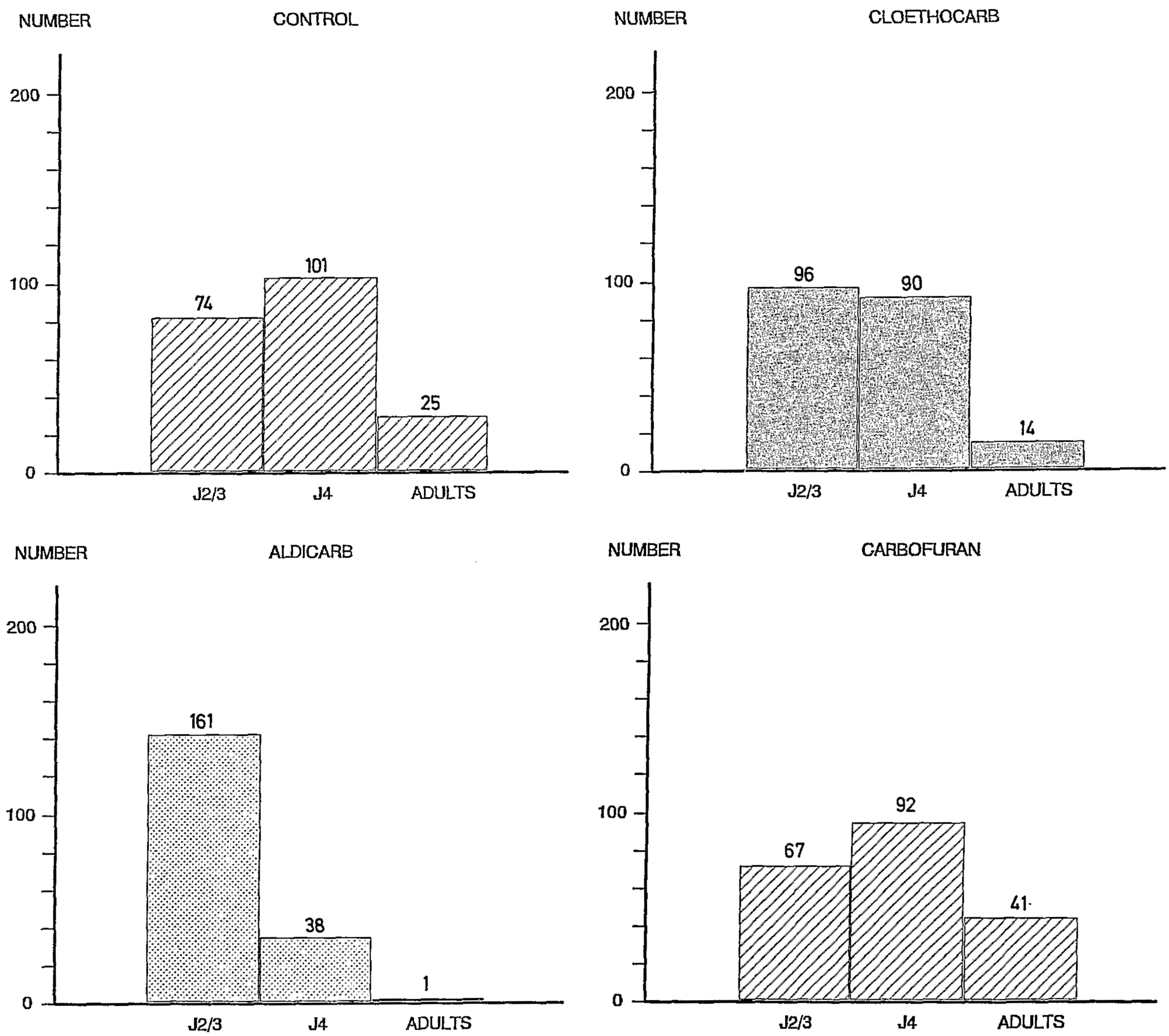

Fig. 2. Frequency distributions of *Heterodera schachtii* developmental stages in untreated sugarbest roots as well as in roots treated with cloethocarb, aldicarb and carbofuran. Distributions significantly different following the χ^2-test are indicated by different bar patterns; mean of eight repetitions.

volume classes from 1-3 and 3-5 $\mu^3 \times 10^5$ and a lower percentage in all remaining classes. The highest percentage of small volume nematodes was caused by aldicarb.

The carbamates affected nematode development (Fig. 2) and nematode growth (Fig. 3) to the same degree. A distribution of volume classes calculated for each *H. schachtii* developmental stage showed that the volume distribution for J2/J3 and J4 was influenced by the carbamates in the same way as the distribution of the developmental stages. On the other hand, the distributions for the volume classes of adult *H. schachtii* did not differ from one another.

Discussion

Carbamate nematicides, which are absorbed by the plant roots and translocated in the plant (Nelmes, Trudgill & Corbett, 1973; Riebel & Beitz, 1976; Steele,

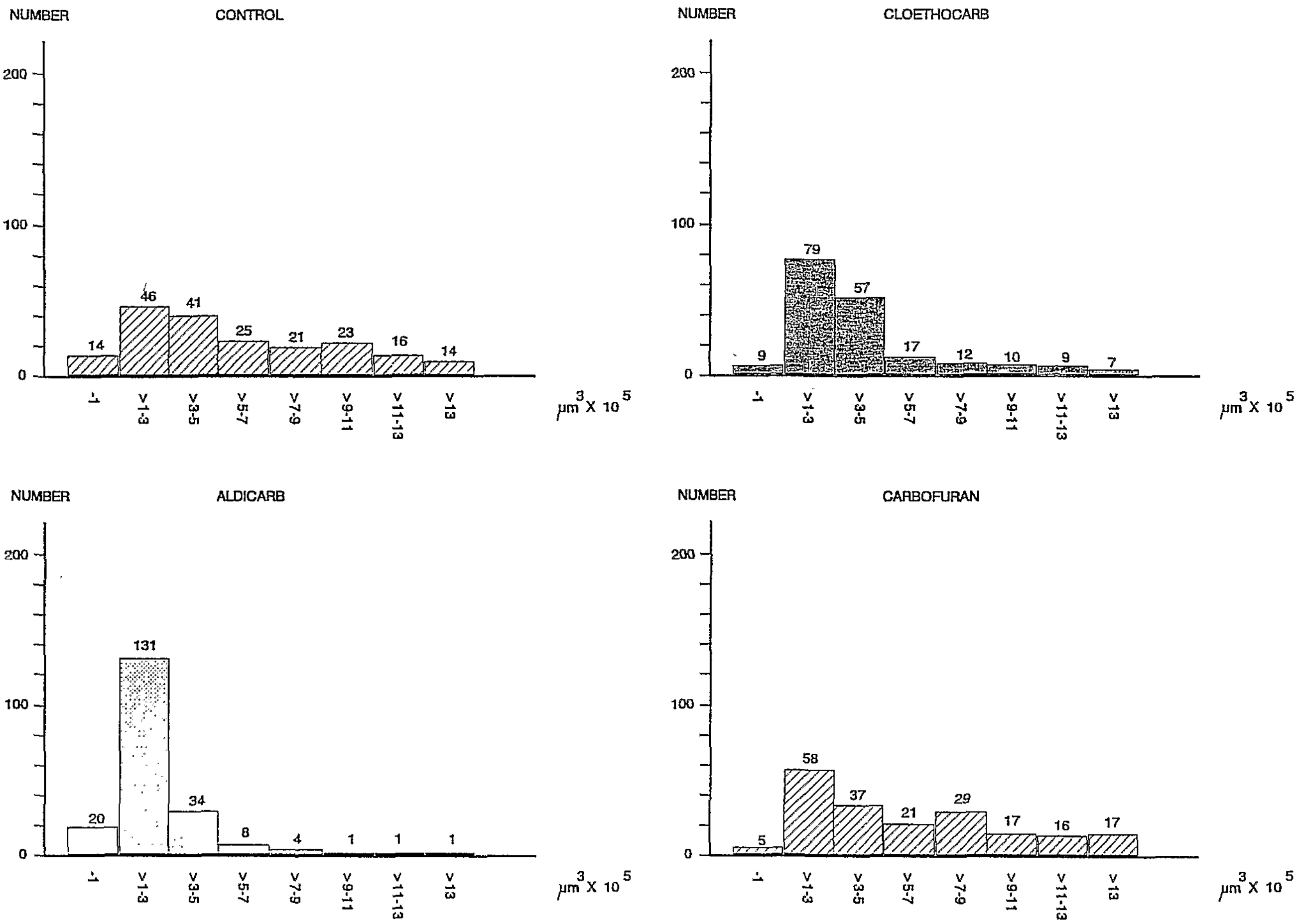

Fig. 3. Frequency distributions of *Heterodera schachtii* volumes of all juvenile stages in untreated sugarbeet roots and well as in roots treated with cloethocarb, aldicarb and carbofuran. (Distributions significantly different following the χ^2-test are indicated by different bar patterns; mean of eight repetitions).

1979; Rouchaud, Moons & Meyer, 1980), trigger processes which alter the composition of substances responsible for nematode attraction to the root or predisposition to root penetration.

Alterations of root attractiveness to *H. schachtii* occurred after sugarbeet treatment with cloethocarb. This carbamate caused a decrease in migration of juveniles to the rhizosphere, whereas, aldicarb and carbofuran did not exhibit this effect. Kerstan and Röpke (1977), however, obtained the opposite results when rape was grown in substrate with 0.1-500 ppm aldicarb. Further investigations are necessary to decide whether carbamate effects on nematode orientation differ with different host plants.

The treatment of sugarbeets with cloethocarb causes juvenile disorientation in the rhizosphere. The disorientation which may be due to a repellant effect or to direct action on the sensory organs of the juveniles, since treatment with this carbamate causes fewer J2 to migrate into sector 5 than with untreated sugarbeets. This disorientation, however, is not absolute, since more nematodes migrated into sector 5 compared to the treatment without sugarbeet.

The exposure of *H. schachtii* juveniles to carbamate concentrations of 0.001-0.1 ppm did not affect penetration of sugarbeet seedlings (Hartwig & Sikora, 1991). We were also unable to influence J2 penetration by treating the plants with aldicarb of carbofuran. Conversely, the application of cloethocarb caused a 14.4 % reduction in penetration. However, the fact that 80.9 % of the J2 that reached the rhizosphere penetrated vs 95.3 % in the control indicates that this mechanism of action is unimportant for control purposes. Kerstan and Röpke (1977) reported that *H. schachtii* juveniles did not penetrate aldicarb treated rape although they were present in the root zone.

Carbamates taken up by plants after nematode penetration also have an influence on juvenile development and growth in the root. This « curative effect » may be the result of direct toxic action on nematode physiology or to disturbances in nematode nutrition through indirect effects on the activity of the syncytium (Steele, 1976). Such carbamate effects would cause developmental disorders in *H. schachtii* and inhibit the growth of juveniles.

In our studies, treatment of sugarbeet seedlings with aldicarb or cloethocarb after *H. schachtii* penetration caused a retardation in nematode development. More nematodes remained in the J2/J3 stages than in the control. The fact that the size of the individual nematode was smaller demonstrates that cloethocarb and aldicarb negatively affect growth whereas carbofuran does not. Similar results were obtained by Steele (1976) with *H. schachtii* and aldicarb. Sugarbeets treated with 5 ppm aldicarb 10 days after penetration of *H. schachtii* had a lower percentage of J3 and J4 than in the control without aldicarb. On the other hand, the percentage of J2 was increased by aldicarb.

Cloethocarb and aldicarb not only delayed development but reduced juvenile growth. Comparison of the body volume of each juvenile stage demonstrated clearly that the J2/J3 and J4 in cloethocarb or aldicarb treated plants were smaller in volume than those in the control. According to Steele (1979) the number of adult *H. schachtii* produced on the root decreases with increasing concentrations of aldicarb in the root tissue. The concentration needed to suppress or retard juvenile development will not be reached in the field at present levels of application. The importance of this mechanism-of-action for plant protection is minimal, because damage to the root occurs mainly through juvenile penetration of the root tissue and not through nematode growth after penetration.

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