Influence of aldicarb, carbofuran, propoxur and fenamiphos on activity of *Pratylenchus penetrans* (Cobb) and *P. crenatus* Loof [1]

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Summary

Aldicarb was the most effective nematicide in reducing the activity of *Pratylenchus penetrans* and *P. crenatus*, followed by fenamiphos, carbofuran and propoxur. Nematode populations which moved down through vertical tubes in 31 hours or less did not show any significant increase or decrease in passage time when nematicide concentrations were increased to 24 µg/ml. A significant interaction existed between temperature, aldicarb concentration, and nematode species. This was interpreted to mean that a dose response to aldicarb was observed for *P. crenatus* at 10° and 20°, but not at 30°, and for *P. penetrans* at 20° and 30°, but not at 10°. Activity of fourth-stage juvenile and adult *P. crenatus* at 22° was inhibited more than the activity of second- or third- stage juveniles when aldicarb concentrations were 6 or 12 µg/ml. Propoxur did not have this effect. There was no significant difference in activity between the various stages of *P. penetrans* when treated with aldicarb or propoxur. Aldicarb treatments of 6 and 12 µg/g dry soil weight reduced numbers of *P. penetrans* and *P. crenatus* in red clover and timothy in the greenhouse more so than equivalent treatments of fenamiphos or propoxur. Propoxur only reduced numbers of *P. crenatus* to a significant degree in red clover. Yield increases were associated with aldicarb treatments on red clover and timothy harbouring *P. penetrans* and *P. crenatus* respectively. Aldicarb at 12 µg/g on red clover harbouring *P. crenatus* also was associated with yield increases. Aldicarb and fenamiphos caused changes in body shape and activity of female nematodes. *Pratylenchus penetrans* recovered more quickly from the effects of fenamiphos than from aldicarb after exposure to solutions of 12 or 24 µg/ml for 24 hours. *Pratylenchus crenatus* recovered more quickly than *P. penetrans* after similar exposure to aldicarb.

Résumé

Influence de l'aldicarbe, du carbofuran, du propoxur et du fenamiphos sur l'activité de Pratylenchus penetrans *(Cobb) et* P. crenatus *Loof*

L'aldicarbe a été le nématicide le plus actif pour réduire l'activité de *Pratylenchus penetrans* et *P. crenatus*, suivi par le fenamiphos, le carbofuran et le propoxur. Le passage de populations de nématodes se déplaçant vers le bas dans des tubes verticaux, en 31 heures, n'était pas significativement ralenti ou accéléré quand les concentrations de nématicide étaient augmentées jusqu'à 24 µg/ml. Une interaction significative a été observée entre la température, la concentration d'aldicarbe et l'espèce du nématode. On considère qu'une réponse à la dose d'aldicarbe est observée pour *P. crenatus* à 10° et 20° mais pas à 30° et pour *P. penetrans* à 20° et 30° mais pas à 10°. Aux doses d'aldicarbe de 6 à 12 µg/ml, l'activité des juvéniles de 4e stade et des adultes de *P. crenatus*, à 22°, était plus inhibée que celle des juvéniles de 2e et 3e stade. Cet effet n'était pas observé avec le propoxur. Aucune différence significative n'a été observée entre les activités des différents stades de *P. penetrans* traités avec l'aldicarbe ou le propoxur. En serre, des traitements à raison de 6 à 12 µg par gramme de sol sec ont réduit le nombre de *P. penetrans* et *P. crenatus* dans le trèfle et la fléole des prés plus que des traitements équivalents avec le fenamiphos ou le propoxur. Ce dernier produit n'a réduit significativement le nombre de *P. crenatus* que dans le trèfle. L'aldicarbe a provoqué des augmentations de rendement chez le trèfle et la fléole des prés hébergeant respectivement *P. penetrans* et *P. crenatus*.

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A la dose de 12 µg/g de sol sec, l'aldicarbe a causé des augmentations de rendement chez le trèfle attaqué par *P. crenatus*. L'aldicarbe et le fenamiphos ont causé des modifications dans la forme du corps et l'activité des nématodes. Chez *P. penetrans*, les effets du fenamiphos se dissipent plus vite que ceux de l'aldicarbe après exposition à des solutions de 12 ou 24 µg/ml pendant 24 heures. Après une exposition similaire avec l'aldicarbe, les effets se sont dissipés plus vite chez *P. crenatus* que chez *P. penetrans*.

Although many of the carbamate and organophosphate pesticides are systemics, their major effect is often as potent contact nematicides against plant-parasitic nematodes in the soil (Bromilow & Lord, 1979 ; Hague, 1979 ; Hague & Pain, 1973 ; Jorgenson, 1978). These compounds usually do not kill plant parasitic nematodes at concentrations used in the field. Instead, they depress populations by interfering with movement and orientation, thereby reducing dispersal, plant invasion and feeding, reproduction, and development (Batterby, 1979 ; Batterby, Le Patourel & Wright, 1977 ; Hough & Thomason, 1975 ; Keetch, 1974 ; McLeod & Khair, 1975 ; Nelmes, 1970 ; Steele & Hodges, 1975).

It is generally accepted that the toxic action of carbamate and organophosphate pesticides is through their ability to inhibit esterases in the nervous system of vertebrates and insects (Corbett, 1974 ; O'Brien *et al.*, 1974). Acetylcholinesterase has been detected in plant-parasitic nematodes (Dickson, Huisingh & Sasser, 1971 ; Rohde, 1960), and the toxic mode is believed to be similar to that in insects (Bunt, 1975 ; Homeyer & Wagner, 1981). However there is no direct evidence of an operational cholinesterase system in nematodes (Van Gundy & McKenry, 1977).

The objective of this study was to determine the effect of three carbamates : aldicarb, carbofuran, and propoxur, and the organophosphate, fenamiphos, on the activity and population size of two common species of rootlesion nematode in eastern Canada, *Pratylenchus penetrans* (Cobb) and *P. crenatus* Loof.

Materials and methods

Populations of *Pratylenchus penetrans* and *P. crenatus* were maintained on Ottawa red clover (*Trifolium pratense* L.) and Climax timothy (*Phleum pratense* L.), respectively. Nematodes were extracted by placing up to 10 g of root in a mist chamber (Seinhorst, 1950) or 50 g of soil in a Baermann pan (Townshend, 1963). Samples then were dried at 100° for 24 hours and counts were expressed as numbers of nematodes per g of dry root or per kg of dry soil. Nematodes used for inoculum were rinsed three times in sterile tap water to reduce microbial contaminants.

The nematicides included in this study were aldicarb 10% granular, carbofuran 1.1% liquid, fenamiphos 1.66% liquid, and propoxur 2% wettable powder. Fresh stock solutions of 100 µg/ml were prepared for each experiment. The nematicides were agitated in sterile tap water for several hours in a shaker, before diluting them to the desired concentrations.

Experimental designs were either randomized blocks or split plots with at least three replicates. Nematode data were transformed to logarithms or arcsines for statistical analyses.

Plastic tubes 3 cm long and 0.7 cm in diameter were covered at one end with nylon mesh and filled to a depth of 2.5 cm with dry sterile sand of particle size 150-250 µm. The tubes were placed vertically in watch glasses so that the mesh interfaced with nematicide solutions containing 0, 3, 6, 12, or 24 µg/ml. It was assumed that most of the nematicide stayed in solution and did not adsorb to the relatively large particles of sterile washed sand (Hough, Thomason & Farmer, 1975). Aliquots of 0.1 ml suspensions of 36.5 $\pm$ 1.9 $S\bar{x}$ *P. penetrans* or 30.6 $\pm$ 1.2 $S\bar{x}$ *P. crenatus* were pipetted onto the surface of the sand in each tube. Tubes were covered with plastic to reduce evaporation and held at 22°. The number of nematodes that moved down the tube into the solution after 31 hours was expressed as a percentage of the total. In addition, the time which allowed 50% of the mobile population to pass through the tubes was calculated from the grouped data by the algorithm of Sampford (1952) and with the assumption of a log normal distribution.

A second experiment recorded the activity of nematodes in the sand columns at 10°, 20°, and 30°, at aldicarb concentrations of 0, 3, and 6 µg/ml. The exposure time was twenty hours and the inoculation levels were 58.3 $\pm$ 1.2 $S\bar{x}$ *P. penetrans* and 42.6 $\pm$ 1.3 $S\bar{x}$ *P. crenatus*.

In the third experiment data were obtained on the number of nematodes that moved down through vertical sand columns in 24 hours at concentrations of 0, 3, 6, and 12 µg/ml of aldicarb and propoxur. The activity of second- and third-stage juveniles was compared to the activity of fourth- stage juveniles and adults. The distinction between categories was based primarily on nematode size and the proportion of each was similar for *P. penetrans* and *P. crenatus*. The inoculation levels were equivalent to the previous experiment and the temperature was 22°.

A greenhouse experiment examined the effect of aldicarb, fenamiphos, and propoxur on population size of *P. penetrans* and *P. crenatus*. Climax timothy or Ottawa red clover were seeded in pots containing 1.0 kg of sterilized Charlottetown fine sandy loam (70% sand, 20% silt, 10% clay). After four days 10 000 nematodes in 50 ml of water were distributed over the surface of each pot and covered with about 1 cm of soil. Seven days later nematicide solutions were added at rates of 0, 6, or 12 µg/g dry soil. Plants were held in the greenhouse in a light-dark regime of 14 and 10 hours, respectively, at a mean temperature of 22°. Nematode counts and foliage weights were recorded eleven weeks after seeding.

The final test consisted of placing five active *P. penetrans* or *P. crenatus* females in 1 ml solutions of aldicarb or fenamiphos at 0, 1, 3, 6, 12, or 24 µg/ml. After 24 hours the nematodes were examined with a stereomicroscope at 70 × for changes in activity or body shape. They were then transferred to sterilized tap water for another 24 hours and re-examined. The procedure used to estimate the amount of coiling or body shrinkage was a modified version of the methods employed by Keetch (1974) and Bunt (1975). The relative scores were assigned on a three-point scale (Snedecor & Cochran 1980). The categories used here, with assigned scores in brackets, were normal sinusoidal motion (1), moderate coiling and decreased movement (2), and severe coiling with immobility and body shrinkage (3). The temperature was 20°.

Results

Aldicarb caused the greatest reduction in the numbers of nematodes passing through the columns within 31 hours followed by fenamiphos, carbofuran, and propoxur (Tab. 1). This trend was similar at 4, 8, and 21 hours and these data were not shown for the sake of brevity. Most of the population was still in the sand columns after 31 hours when aldicarb concentrations were 24 µg/ml. For the active population which included only those nematodes which moved down the columns in 31 hours or less, there was no definite trend in the amount of passage time as nematicide concentrations increased. In some cases the passage time increased as the nematicide concentrations increased, while in other instances there was no change or the opposite occurred.

The statistical analyses showed that, in addition to the main effects of temperature and nematicide concentration, a significant second-order interaction existed between temperature, aldicarb concentration and nematode species. This indicated that a dose response to aldicarb was observed for *P. crenatus* at 10° and 20° but not at 30°, and for *P. penetrans* at 20° and 30° but not at 10° (Fig. 1).

Aldicarb at concentrations of 6 and 12 µg/ml reduced the activity of *P. penetrans*, while the movement of *P. crenatus* was lessened at 12 µg/ml (Tab. 2). Fourth-stage juveniles and adults of *P. crenatus* were affected more than second- or third-stage juveniles. Numerically the trend was opposite for *P. penetrans* but was not statistically significant. Propoxur did not significantly affect the activity of *P. penetrans* at any of the concentrations. However, the mobility of *P. crenatus* was reduced as propoxur concentrations were increased, but there was no significant difference between the stages.

Aldicarb reduced the numbers of both nematode species in the greenhouse pots except for the 6 µg/g level in timothy pots inoculated with *P. penetrans* (Tab. 3). The same trend in red clover occurred for fenamiphos, though the reductions were not as marked as in the aldicarb treatments. Propoxur at 12 µg/g reduced the population size of *P. crenatus* in red clover but to a lesser degree than aldicarb. The population size of *P. penetrans* was not reduced significantly by propoxur in red clover and timothy.

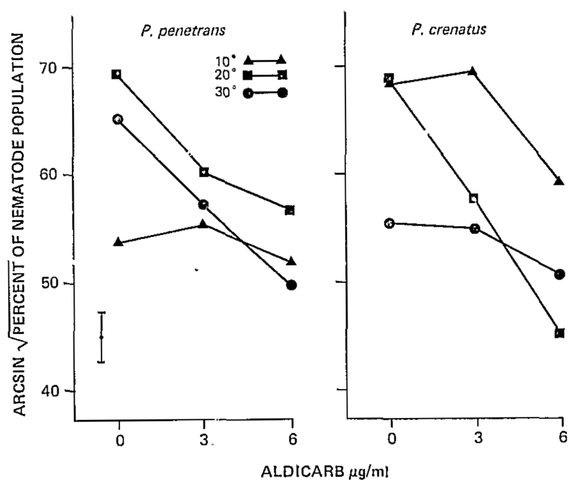

Fig. 1. Arcsin $\sqrt{\text{percent}}$ of populations of *Pratylenchus penetrans* and *P. crenatus* moving within 20 hours at 10°, 20°, or 30° down vertical sand columns treated with aldicarb. Vertical bar represents the standard error at P = 0.05 and n = 4.

Aldicarb treatments were associated with both an increase in yield and a decrease in numbers of *P. penetrans* and *P. crenatus* in red clover and timothy, respectively. The aldicarb treatment of 12 µg/g also resulted in an increase in yield of red clover when *P. crenatus* numbers were reduced. The only other significant yield increases were for fenamiphos at 12 µg/g in red clover and timothy pots that contained *P. penetrans* and *P. crenatus*, respectively.

Both aldicarb and fenamiphos caused significant changes in nematode activity and body shape (Table 4). *Pratylenchus penetrans* appeared to recover from the effects of fenamiphos earlier than from aldicarb after being exposed to concentrations of 12 and 24 µg/ml for 24 hours. Also, *P. crenatus* recovered more fully than *P. penetrans* from the effects of aldicarb at concentrations of 12 and 24 µg/ml.

Table 1

Percentage of the total population at 31 hours which moved down through 2.5 cm vertical soil columns treated with nematicides, and the number of hours (1) required for 50 % of the active population to move through the same columns

Nematicide	*Nematode species* (2)	*Nematicide concentrations (µg/ml)*				
		0	*3*	*6*	*12*	*24*
Total Population (3) *(percent)*						
Aldicarb	PP	95.1 ± 2.3 (4)	80.7 ± 3.7	41.1 ± 6.0	30.6 ± 2.6	13.0 ± 2.1
	PC	89.1 ± 4.2	88.1 ± 7.2	58.8 ± 8.2	50.3 ± 3.6	14.3 ± 2.8
Fenamiphos	PP	94.2 ± 2.4	80.2 ± 4.0	61.6 ± 5.0	68.0 ± 7.4	17.5 ± 5.1
	PC	88.0 ± 5.7	89.8 ± 4.9	85.8 ± 5.8	50.3 ± 3.0	36.1 ± 8.0
Carbofuran	PP	92.0 ± 1.8	83.6 ± 4.5	66.3 ± 10.4	59.5 ± 7.4	42.7 ± 7.7
	PC	89.6 ± 1.7	84.6 ± 3.0	71.5 ± 3.1	70.2 ± 8.0	39.8 ± 9.3
Propoxur	PP	93.1 ± 2.8	88.1 ± 3.3	71.3 ± 4.1	80.6 ± 3.2	61.8 ± 4.0
	PC	89.8 ± 4.4	83.8 ± 4.5	76.5 ± 4.1	68.8 ± 3.5	68.0 ± 9.4
Active Population (3) *(no. of hours)*						
Aldicarb	PP	4.8 ± 0.4 (4)	4.7 ± 0.2	5.1 ± 0.3	6.3 ± 0.4	6.1 ± 0.3
	PC	6.9 ± 0.4	7.0 ± 0.6	5.9 ± 0.3	5.7 ± 0.3	—(5)
Fenamiphos	PP	4.9 ± 0.4	4.6 ± 0.2	5.0 ± 0.4	4.9 ± 0.3	4.9 ± 0.4
	PC	6.3 ± 0.3	6.1 ± 0.2	5.7 ± 0.2	5.2 ± 0.5	4.4 ± 0.2
Carbofuran	PP	4.4 ± 0.3	4.1 ± 0.2	4.1 ± 0.7	4.4 ± 0.2	4.5 ± 0.3
	PC	6.2 ± 0.3	6.1 ± 0.5	6.0 ± 0.4	7.6 ± 1.0	7.7 ± 1.1
Propoxur	PP	4.4 ± 0.3	4.7 ± 0.2	4.5 ± 0.2	3.7 ± 0.4	3.0 ± 0.8
	PC	6.7 ± 0.3	7.8 ± 0.5	7.2 ± 0.7	7.5 ± 0.7	8.3 ± 0.6

(1) Observation times were 4, 8, 21, and 31 hours after nematodes were added to columns.

(2) PP = *Pratylenchus penetrans*, PC = *P. crenatus*.

(3) Total population includes those nematodes left in the columns after 31 hours ; active population represents only those nematodes which passed through the columns in 31 hours or less.

(4) Mean of four replicates ; standard errors may be compared within each population.

(5) Not enough specimens.

Table 2

Percentages (1) of populations of second- and third-stage juveniles (2 + 3) and fourth-stage juveniles and adults (4 + A) moving down 2.5 cm vertical sand columns treated with aldicarb or propoxur

Nematicide	*Nematode species*	*Category*	*Nematicide concentration (µg/ml)*			
			0	*3*	*6*	*12*
Aldicarb	*P. penetrans*	2 + 3	68.7 (2)	66.2	41.3	32.1
		4 + A	75.4	69.2	51.3	41.2
	P. crenatus	2 + 3	68.3	66.4	61.9	46.0
		4 + A	70.3	51.8	44.9	31.2
Propoxur	*P. penetrans*	2 + 3	77.6	72.1	72.5	74.7
		4 + A	78.7	75.4	73.8	67.0
	P. crenatus	2 + 3	74.2	72.3	54.9	38.4
		4 + A	76.3	62.4	49.0	35.6

(1) Arcsin transformation ; counts were done 24 hours after nematodes were added to the tops of the sand columns; temperature was 22°.

(2) Mean of four replicates ; standard error of a mean derived from the error mean square = ± 5.2.

Table 3

Effect of nematicide treatments on populations of *Pratylenchus penetrans* (PP) and *P. crenatus* (PC), and on foliage yields (1)

Host plant	*Nematode species*	*Nematicide treatments* (2)						
		0	A_6	A_{12}	F_6	F_{12}	P_6	P_{12}
No. of nematodes/pot (× 100)								
Red clover	PP	72a (3)	8c	7c	23bc	15bc	32ab	29ab
	PC	83a	11bc	7c	16bc	17bc	19bc	23b
Timothy	PP	28a	13ab	10b	17ab	12ab	27a	17ab
	PC	41a	14bc	8bc	17ab	20ab	20ab	16ab
Foliage yield in grams/pot								
Red clover	PP	1.63 (4)	2.47	2.47	2.20	2.30	1.93	2.17
	PC	1.63	2.17	2.37	1.50	1.87	1.63	2.13
Timothy	PP	5.07	5.03	5.52	4.97	5.55	5.01	4.88
	PC	4.11	4.93	5.08	4.51	4.83	4.63	4.38

(1) 10 000 nematodes added to each pot 4 days after seeding ; nematicides applied seven days later ; nematode and yield data obtained eleven weeks after seeding.

(2) Aldicarb (A), fenamiphos (F), and propoxur (P) applied at 6 and 12 µg/g in relation to the soil weight; *O* is the check.

(3) Geometric mean of three replicates ; numbers in horizontal rows followed by the same letter are not significantly different according to Duncan's multiple range test at P = 0.05.

(4) Arithmetic mean of the dry weight of three replicates ; standard error of a mean equals ± 0.21.

Table 4

Effect of aldicarb and fenamiphos on activity and body shape (1) of female nematodes after 24 hours of exposure, and degree of recovery after 24 hours in water

Nematode species	*Treatment*	*Nematicide concentration (µg/ml)*					
		0	*1*	*3*	*6*	*12*	*24*
Nematicide							
P. penetrans	Aldicarb	1.00 (2)	2.40	2.53	2.73	2.93	2.87
	Fenamiphos	1.00	1.53	2.20	2.93	3.00	3.00
P. crenatus	Aldicarb	1.27	1.87	2.53	2.73	2.80	3.00
	Fenamiphos	1.13	1.40	2.13	2.60	2.87	3.00
Water							
P. penetrans	Aldicarb	1.00	1.20	1.27	1.20	2.47	2.53
	Fenamiphos	1.00	1.27	1.53	1.53	2.13	1.93
P. crenatus	Aldicarb	1.13	1.13	1.67	1.60	1.87	2.07
	Fenamiphos	1.07	1.40	1.53	2.07	1.80	1.80
Difference (3)							
P. penetrans	Aldicarb	0.00	1.20	1.26	1.53	0.46	0.34
	Fenamiphos	0.00	0.26	0.67	1.40	0.87	1.07
P. crenatus	Aldicarb	0.14	0.74	0.86	1.13	0.93	0.93
	Fenamiphos	0.06	0.00	0.60	0.53	1.07	1.20

(1) Assigned relative scores (Snedecor & Cochran, 1980) ; normal sinusoidal motion = 1.00, modest body coiling with some decrease in motion = 2.00, severe body coiling, immobility, and shrinkage = 3.00. Five females in each replicate.

(2) Mean of three replicates ; standard error of a mean for Nematicide, Water, and Difference categories are $\pm$ 0.08, $\pm$ 0.12, and $\pm$ 0.14, respectively.

(3) Indicates the relative change or reduction in body coiling and shrinkage when nematodes were removed from the nematicide solution, and washed in water for 24 hours.

Discussion

Aldicarb caused the greatest reduction in nematode activity, fenamiphos and carbofuran were somewhat intermediate, while propoxur had the least effect (Tab. 1, 3, 4). This trend agreed closely with the acute oral LD50s for rats reported by Martin and Worthing (1977) at 0.9, 8.0-14.0, 15.3-19.4, and 90.0-128.0 mg of pesticide per kg of rat weight for aldicarb, carbofuran, fenamiphos, and propoxur, respectively. The similarity is interesting since nematodes do not possess a respiratory or circulatory system and would not be subject to the same dysfunction as in rats.

Previous work showed aldicarb to be more effective than fenamiphos in the control of *Globodera rostochiensis* (Woll.) under field conditions (Whitehead *et al.*, 1973 ; Moss, Crump & Whitehead, 1975). Brodie and Good (1973) observed that less aldicarb than carbofuran or fenamiphos was required for control of *Meloidogyne incognita* (Kofoid & White), the latter two pesticides having about the same effect. However, Marban-Mendoza and Viglierchio (1980) needed carbofuran solutions which were 100 times the strength of fenamiphos solutions to immobilize *Pratylenchus vulnus* Allen & Jensen. Propoxur was shown previously to be somewhat innocuous as a nematicide (Bunt, 1975).

There was little clear indication that some nematodes were less affected by nematicides since the time required for 50% of the active population to move through the columns in 31 hours or less increased in some instances and decreased in others as the nematicide concentrations increased (Tab. 1). The increase in nematode activity as concentrations of nematicides increased was due probably to rapid body contractions. For example propoxur has a rapid knock-down effect on flying insects caused by inhibition of acetylcholinesterase (Martin & Worthing, 1977), and a similar action causing rapid body contractions in *P. penetrans* could be responsible for the increase in activity for a short time.

The influence of temperature on aldicarb (Fig. 1) was not unexpected since this phenomenon is common for many pesticides (Bunt, 1975). The differing dose response to aldicarb is related to the temperature of the two species, in which *P. penetrans* generally lives at higher temperatures than *P. crenatus* (Kimpinski & Willis, 1981). Aldicarb therefore was not as effective on *P. penetrans* at 10°, or on *P. crenatus* at 30°.

Adults and fourth-stage juveniles of *P. crenatus* were less active than the younger stages when aldicarb was present. The situation was reversed for *P. penetrans* though the differences were only significant at $P = 0.1$ (Tab. 2). Previous reports indicated that immature stages were more susceptible than adults to nematicides (Evans & Thomason, 1971 ; Marban-Mendoza & Viglierchio, 1980). Fourth-stage juveniles and adults of *P. penetrans* invaded alfalfa more readily than second- and third-stage juveniles (Sontirat & Chapman, 1970 ; Townshend, 1978), which suggests that older stages are more active. Active nematode species metabolize and eliminate nematicides more rapidly than less active species (Batterby, Le Patourel & Wright, 1977 ; Marks, Thomason & Castro, 1968), and the same process may apply to different stages within a species. Unfortunately, information is lacking on the activity of the different stages of *P. crenatus*.

The reason for the better recovery of *P. crenatus* as compared to *P. penetrans* from the effects of aldicarb (Tab. 4) may be related to cholinesterase concentrations in the nematodes. However, more information is needed on the biochemistry of plant-parasitic nematodes before major advances can be made in understanding the action and selectivity of nematicides (Marks, 1971 ; Wright, 1981). Voss (1981) reasoned that the use of acetylcholinesterase inhibiting insecticides with high specificity to closely related species was limited, though families and orders could be separated. There may be a better chance to develop nematicides with appropriate selectivity since agricultural practices usually favour only a few nematode species, often from different families.

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