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Effect of long term aldicarb applications on the development of field populations of some endoparasitic nematodes

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Summary – *Globodera rostochiensis, Meloidogyne naasi*, and *Pratylenchus crenatus* multiplying on potato, wheat, and maize, respectively, were annually exposed for 15 consecutive years to aldicarb (0, 0.3, or 0.6 g/m^2). In the sixteenth year, all of the previously exposed and unexposed populations were treated (0, 0.3, or 0.6 g/m^2) with the same nematicide. The development of nematodes inside the host roots was used to assess the nematode response to the final treatment. This response varied with the nematicide applications and with the nematode stages considered. Depending on the nematode species and degree of exposure to nematicide, long term annual aldicarb treatments reduced or increased the presence of some nematode stages of the three species. The effects, however, were not constant and not consistent from one stage to another. © Elsevier - ORSTOM

Résumé – ***Effets d'applications à long terme d'aldicarbe sur le développement en champ de populations de nématodes endoparasites*** - *Globodera rostochiensis, Meloidogyne naasi* et *Pratylenchus crenatus* multipliés sur, respectivement, pomme de terre, blé et maïs ont été soumis pendant 15 années consécutives à des applications d'aldicarbe aux doses de 0 (témoin), 0,3 et 0,6 g/m^2. Lors de la seizième année, les populations témoins et traitées ont reçu le même traitement que précédemment (0 [témoin], 0,3 et 0,6 g/m^2), avec le même nématicide. Le développement des nématodes dans les racines de l'hôte est utilisé pour vérifier la réaction à ce traitement final. Cette réaction varie en fonction des doses de nématicide et des stades des trois nématodes. Suivant l'espèce de nématode et l'exposition au nématicide, les traitements à long terme à l'aldicarbe diminuent ou accroissent la présence de certains stades des trois espèces. Ces effets, cependant, ne sont ni constants ni continus d'un stade à l'autre. © Elsevier - ORSTOM

Keywords: aldicarb, effects, *Globodera rostochiensis*, *Meloidogyne naasi*, nematode development, *Pratylenchus crenatus*.

Since the early 1960s, non-fumigant organophosphate and carbamate compounds have been developed which are active against plant-parasitic nematodes. In general, these nematicides depress but do not eliminate nematode populations. Until the early 1980s, no cases of resistance to these nematicides were found among field populations of plant-parasitic nematodes (Wright, 1981).

Greenhouse and laboratory trials exposing to nematicides both nematicide-stressed and non-stressed populations of *Xiphinema index*, *Meloidogyne incognita*, and *Pratylenchus vulnus* suggested that resistance to non-fumigant nematicides had developed in certain stressed and unstressed populations (Yamashita & Viglierchio, 1986*a*, *b*, *c*, 1987; Yamashita *et al.*, 1986). Field resistance seemed to have appeared in *X. index* and *M. incognita* populations after treatments for 3 consecutive years with either carbofuran or phenamiphos (Yamashita & Viglierchio, 1987).

In 1976, Dr Coolen, former nematologist at the State Research Station of Nematology, initiated an experiment designed to study the effects on *Globodera rostochiensis*, *Meloidogyne naasi*, and *Pratylenchus crenatus* field populations of annual applications of aldicarb made during a long period. This paper presents data obtained during the sixteenth year of the experiment.

Materials and methods

NEMATODES

The experiment was conducted on 27 microplots (1.5 × 4 m) established in 1976 on a site with sandy loam soil at pH 5.17 free of *G. rostochiensis*, *M. naasi*, and *P. crenatus*. Nine microplots were equally infested with field populations of either *G. rostochiensis*, *M. naasi*, or *P. crenatus*. The nematodes were collected from experimental fields which had not previously been in contact with any nematicide. During the experiment, potato, wheat, and maize were used as host for *G. rostochiensis*, *M. naasi*, and *P. crenatus*, respectively. These crops were grown according to good agricultural practices.

NEMATICIDAL TREATMENTS

For 15 consecutive years, aldicarb was applied once each year, at planting or sowing of the host crop. The nine plots for each nematode species received either 0, 0.3, or 0.6 g/m^2 aldicarb (three replicates per dose). The treated and untreated populations are called "nematicide-exposed" and "wildtype" populations, respectively. During the sixteenth year (1991), each plot (either previously treated or untreated) was divided into three equal sub-plots, which then received

either 0, 0.3, or 0.6 g/m^2 aldicarb. The aldicarb in each treatment was broadcast and incorporated into the soil. For easier reading of this paper, sub-plots and their populations are named according to both the exposure they underwent during the first 15 years and the treatment they received during the sixteenth year. For example, control sub-plots and populations are designated as 0-0 plots/populations, and subplots/populations being exposed to 0.3 g/m^2 aldicarb for 15 years and treated with 0.6 g/m^2 aldicarb in 1991 are named 0.3-0.6 sub-plots/populations. x is used when both dosages, 0.3 or 0.6 g/m^2 aldicarb, can be considered together: x-0 populations designate populations which were exposed to 0.3 or 0.6 g/m^2 aldicarb for 15 years and not treated in the final year.

Observations

The development of nematodes inside their host roots was used to assess nematode response to the final nematicidal treatment. During the growing season, the nematode population present in the host roots of each sub-plot (three replicates) was sampled twice on different occasions (Tables 1-3). Each subplot was sampled separately. The nematodes were extracted by the method described by Coolen (1979).

Statistics

All data were analysed following a *ln(x+1)* transformation. Mean comparisons were evaluated by Duncan's multiple range test with a significance level of 5%.

Results

At the start of the 1991 season, no significant differences were observed for each of the nematode species between the various preplant soil populations obtained after fifteen annual nematicide applications (data not shown). The mean population density per 100 g soil was 4455 ± 736 eggs and second stage juveniles, 480 ± 120 second stage juveniles, and 2450 ± 572 individuals for *G. rostochiensis*, *M. naasi*, and *P. crenatus*, respectively.

Responses of nematodes to nematicide applications following extended exposures to nematicides can be evaluated by different criteria. In this paper we applied the evaluation criteria used by Yamashita *et al.* (1986).

Effects of nematicide exposure on nematode reproduction

The isolated effects of long term nematicide exposure on nematode reproduction can be evaluated by comparing nematode numbers in populations of untreated wild-types (0-0 populations, not in contact with nematicides at any time) and those in previously exposed population left untreated in the final year (x-0 populations).

G. rostochiensis: At the first sampling in May 1991, the numbers of males found in 0.3-0 and 0.6-0 populations (20 and 13) were significantly lower than in 0-0 population (53) (Table 1). Significant differences in numbers of females were found between 0.6-0 populations (160) and 0-0 populations (467). There were no significant differences at the second sampling in July 1991.

M. naasi: There were no significant differences between the 0.3-0 and 0.6-0 populations and 0-0 population at the first sampling (Table 2). At the second sampling, the numbers of females in 0.6-0 and 0.3-0 populations (6000 and 3333) were significantly larger than in 0-0 population (1033).

P. crenatus: Significant differences between x-0 and 0-0 populations were detected only at the first sampling (Table 3). The nematode numbers in 0.6-0 populations (24617) were significantly lower than in 0-0 population (62083).

Increased susceptibility of previously exposed populations to a further nematicide application

According to Yamashita *et al.* (1986), increased susceptibility can be inferred when presently-treated previously-exposed population (x-x populations) levels are lower than the population level of: *i*) nematicide treated wild-type population (0-x populations), *ii*) wild-type population control (0-0 population), and *iii*) exposed populations control (x-0 populations). Of the three comparisons, only the first two require significant differences as evidence of increased susceptibility to the nematicide.

G. rostochiensis: Based on the above-mentioned criteria, an increased susceptibility was observed at the second sampling in exposed swollen stages and females. The number of swollen stages was significantly smaller in 0.6-0.3 populations (0) than in 0-0.3 populations (33), 0-0 population (140), and 0.6-0 population (33). The same pattern was observed with females: the numbers of females in 0.6-0.3 populations (0) were lower than in 0-0.3 populations (60), 0-0 populations (353), and 0.6-0 population (106; Table I).

Meloidogyne naasi: There was no increased susceptibility in all developmental stages at both samplings.

P. crenatus: At the second sampling, it was observed that previous exposure to 0.6 g/m^2 aldicarb increased the susceptibility of *P. crenatus* to a 0.3 g/m^2 aldicarb treatment. Nematode numbers in 0.6-0.3 populations (520) were significantly lower than in 0-0.3 populations (2560), 0-0 populations (86 400), and 0.6-0 populations (90 200; Table 3).

Table 1. *Mean number of different stages of* Globodera rostochiensis *found in 5 g potato root tissue at two sampling dates.*

Sampling date	Developmental stage	Aldicarb dose (g/m^2)			
		before 1991	in 1991		
			0.6	0.3	0
30.05.1991	Second stage juveniles	0.6	7 *b*	0 *b*	727 *a*
		0.3	7 *b*	20 *b*	520 *a*
		0	0 *b*	6 *b*	2313 *a*
	Swollen juvenile stages	0.6	13 *b*	33 *b*	2240 *a*
		0.3	7 *b*	0 *b*	1420 *a*
		0	0 *b*	0 *b*	3793 *a*
	Females	0.6	0 *c*	0 *c*	160 *b*
		0.3	0 *c*	0 *c*	293 *a*
		0	0 *c*	0 *c*	467*a*
	Males	0.6	0 *b*	0 *b*	20 *b*
		0.3	0 *b*	0 *b*	13 *b*
		0	0 *b*	0 *b*	53 *a*
16.07.1991	Second stage juveniles	0.6	7 *a*	8 *a*	13 *a*
		0.3	20 *a*	47*a*	53 *a*
		0	33 *a*	72*a*	40 *a*
	Swollen juvenile stages	0.6	0 *c*	0 *c*	33 *ab*
		0.3	13 *bc*	13 *bc*	33 *ab*
		0	0 *c*	33 *ab*	140 *a*
	Females	0.6	0 *d*	0 *d*	106*ab*
		0.3	0 *d*	20 *cd*	186 *a*
		0	0 *d*	60 *bc*	353 *a*
	Males	0.6	7 *b*	0 *b*	73 *a*
		0.3	27 *ab*	53 *ab*	27 *ab*
		0	20 *b*	33 *ab*	147 *a*

Per sampling date and stage, numbers followed by the same letter are not significantly different according to the Duncan test ($P \leq 0.05$).

INDIFFERENT RESPONSE TO NEMATICIDE APPLICATIONS

An indifferent response to nematicide applications can be interpreted as a development of resistance. When treatment decreases a wild-type population (0-x) to a level below that of the wild-type population control (0-0), a similar effect might be expected in exposed populations (x-0). However, if an exposed population (x-x) is not comparably reduced to below the level of an exposed population control (x-0), resistance to the nematicide may be inferred (Yamashita *et al.*, 1986).

G. rostochiensis: At the first sampling, the nematicide-exposed males showed an indifferent response. The number of males (0 and 0) was significantly lower in 0-x populations than in 0-0 populations (53; Table 1). Male production in x-x populations, however, was not significantly different from that in 0.6-0 and 0.3-0 populations (four 0 values vs. 20 and 13). At the second sampling, an indifferent response was observed for the swollen stages and the males. Aldicarb treatments (0.6 g/m^2) significantly reduced these stages in the wild-type population (0 vs. 140 and 20 vs. 147 for swollen stages and males, respectively). The same

Table 2. *Mean number of different stages of* Meloidogyne naasi *found in 5 g wheat root tissue at two sampling dates.*

Sampling date	Developmental stage	Aldicarb dose (g/m^2)			
		before 1991	in 1991		
			0.6	0.3	0
06.06.1991	Second stage juveniles	0.6	673 *d*	906 *cd*	2220 *abc*
		0.3	987 *bcd*	3067 *ab*	4467 *a*
		0	133 *e*	800 *cd*	1493 *abcd*
	Swollen juvenile stages	0.6	0 *b*	0 *b*	773 *a*
		0.3	13 *b*	13 *b*	2333 *a*
		0	13 *b*	0 *b*	347*a*
23.07.1991	Second stage juveniles	0.6	40 *b*	133 *b*	2333 *a*
		0.3	33 *b*	7 *b*	8000 *a*
		0	7 *b*	93 *b*	3333 *a*
	Females	0.6	27 *c*	47 *c*	6000 *a*
		0.3	20 *c*	100 *c*	3333 *a*
		0	0 *d*	20 *c*	1033 *b*
	Eggs	0.6	813 *c*	8580 *bc*	450 000 *a*
		0.3	1227 *c*	3867 *c*	403 333 *a*
		0	193 *d*	4427 *c*	93 333 *ab*

Per sampling date and stage, numbers followed by the same letter are not significantly different according to the Duncan test ($P \leq 0.05$).

treatments, however, did not influence significantly the 0.3 g/m^2 aldicarb exposed populations (13 vs. 33 and 27 vs. 27 for swollen stages and males, respectively).

M. naasi and *P. crenatus:* none of the stages of these two nematodes showed an indifferent response at both samplings.

Larger nematode numbers in previously exposed populations than in wild-type populations

An increase in nematode numbers following nematicide treatment of previously exposed populations (x-x) greater than the nematode number increase following treatment of wild-type populations (0-x) would provide evidence of the development of resistance in the exposed population.

G. rostochiensis and *P. crenatus:* With regard to the larger population effect, no significant differences were observed for these species.

M. naasi: Aldicarb treatment (0.6 g/m^2 aldicarb) of populations previously exposed to nematicide (x-0.6), produced, at the first sampling, a significantly larger number of second stage juveniles (673 and 987) than did wild-type (0-0.6) populations (133; Table 2). The 0.3 g/m^2 aldicarb treatment apparently induced resistance as observed after a 0.3 g/m^2 aldicarb exposure (0.3-0.3 vs. 0-0.3; 3067 vs. 800). At the second sampling, the egg number found after a 0.6 g/m^2 aldicarb

Table 3. *Mean number of* Pratylenchus crenatus *individuals found in 5 g maize root tissue at two sampling dates.*

Sampling date	Aldicarb dose (g/m^2)			
	before	in 1991		
	1991	0.6	0.3	0
06.06.91	0.6	139 *de*	1667 *c*	24 617 *b*
	0.3	108 *e*	317 *cd*	39 538 *ab*
	0	172 *de*	603 *cd*	62 083 *a*
23.07.91	0.6	600 *c*	520 *c*	90 200 *a*
	0.3	1020 *bc*	4740 *b*	80 200 *a*
	0	1180 *bc*	2560 *b*	86 400 *a*

Per sampling date and stage, numbers followed by the same letter are not significantly different according to the Duncan test ($P \leq 0.05$).

treatment was significantly larger in x-0.6 populations than in the 0-0.6 population (813 and 1227 vs. 193).

Discussion

Our results show that long term aldicarb applications affected the development of *G. rostochiensis*, *M. naasi*, and *P. crenatus* inside their host roots. Different kinds of effects on the developmental stages were observed: *i*) lower population levels in x-0 populations than in x-x populations; *ii*) increased susceptibility in x-x populations; *iii*) resistance phenomena as expressed by indifference to nematicides; and *iv*) resistance as demonstrated by larger population levels in x-x populations. These effects were influenced by the stage of the nematode, the exposure dose, and the time of observation. The effects, however, were not constant and not consistent from one stage to another. If any effects were present in adult stages, they were nearly always of the non resistance-type. Resistance in adults was only observed with males of *G. rostochiensis* (indifferent response at both samplings).

In our experiment, selective pressures, required for the development of non-fumigant nematicide resistance under field conditions, were apparently present. However, at the end of a series of fifteen consecutive annual aldicarb applications, differences between wild-type and nematicide-exposed populations were not significant. Therefore, it can be concluded that the required degree of selective pressures (Wright, 1981) was absent. The buffer effect of the soil probably contributed to this result. In the field, part of the nematode population will likely lie out of the zone of action of the nematicide. Nematicides are seldom equally distributed throughout the soil profile and it is probable that nematodes are not all exposed to the same rate of nematicide. Moreover, aldicarb can leach to deeper soil layers, depending on climatic conditions (Smelt & Leistra, 1992).

The influence of soil-inhabiting micro-organisms on pesticide degradation is another factor which reduces nematicide efficacy in soil (Smelt & Leistra, 1992). Occasionally, accelerated degradation develops after only one application, as observed by Harris *et al.* (1984). However, this may not occur with other combinations of nematicide and soil, even after a long series of applications. It appears unlikely that accelerated degradation occurred in our experiment: if it had occurred, it would have neutralised, at least partially, the nematicide treatments in the sixteenth year.

Yamashita and Viglierchio (1987*a*) were able to demonstrate the development of field resistance to non-fumigant nematicides in *X. index*. The results of their tests suggested that the development of non-fumigant nematicide resistance under field conditions does not require a continuous monthly exposure regimen. Apparently, in the vineyard used for their tests, sufficient selective pressures were available to enable resistance to develop. The authors suggested that the development of resistance may vary with the specific host, nematode species and strain, nematicide, soil types, and related factors. An important difference between their experiment and ours is found in the perennial character of the vineyard, which authorizes a continuous multiplication of the nematode.

In the last 8 years, no reports of field resistance were published. Apparently, if resistance does appear, it is very scarce. Resistance may be masked by opposing forces. As Yamashita and Viglierchio (1987*a*) suggest, nematode selection for resistance may not necessarily evolve concomitant qualities of fitness for the environment. The same authors also accept a low reproductive potential of treated populations as one of the possible masking effects. Our experiment, however, clearly demonstrates that long term nematicide applications do not influence the reproductive potential of *G. rostochiensis, M. naasi*, and *P. crenatus*.

Some of the criteria for demonstrating resistance advocated by Yamashita *et al.* (1986) and used in this paper may not be sufficiently rigorous to sustain the conclusion for evidence for resistance. The recording of nematode numbers greater in exposed populations than in wild-type populations is seen as a development of resistance. This effect was observed only in *M. naasi*. This behaviour, however, could be explained by the effect of a natural mechanism causing biological control in the absence of a nematicide treatment. In presence of such a treatment, the natural control may have been removed, allowing a greater survival of second stage juveniles. Further experimental work in which more factors can be controlled is needed.

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