Induction of short-term tolerance to nonfumigant nematicides in wild populations of *Xiphinema index* and *Pratylenchus vulnus*

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SUMMARY

Wild populations of *Xiphinema index* and *Pratylenchus vulnus* were tested for their ability to tolerate various concentrations of carbofuran, oxamyl and phenamiphos. Then, the same nematode cultures were treated with subnematicidal levels of either carbofuran, oxamyl or phenamiphos (induction treatment). On various days following induction treatment the nematodes were again tested for their tolerance to the three nonfumigant nematicides (NFN). In almost all cases pretreatment of the nematodes with subnematicidal levels of a NFN induced increased tolerance to subsequent nematicidal-level exposures. The degree of this effect varied with the nematode species and the specific NFN.

RÉSUMÉ

Induction d'une tolérance à court terme aux nématicides non fumigants chez des populations sauvages de Xiphinema index *et* Pratylenchus vulnus

Des populations sauvages de *Xiphinema index* et de *Pratylenchus vulnus* ont été testées pour leur capacité à tolérer diverses concentrations de carbofuran, d'oxamyl et de phenamiphos. Des nématodes provenant des mêmes élevages ont été ensuite traités avec des doses subnématicides de carbofuran, d'oxamyl ou de phénamiphos (traitement d'induction). A des doses variables après ce traitement d'induction, les nématodes ont été de nouveau testés pour leur résistance aux trois nématicides non fumigants (NNF) cités. Dans la plupart des cas, le prétraitement des nématodes avec des taux subnématicides d'un NNF induit une tolérance lors du traitement consécutif à un taux nématicide. Le degré de cet effet varie avec l'espèce du nématode et le NNF considérés.

Greenhouse trials have been conducted with various populations of *Xiphinema index*, *Meloidogyne incognita* and *Pratylenchus vulnus* (Yamashita & Viglierchio, 1986*a*, 1986*b*; Yamashita, Viglierchio & Schmitt, 1986). A portion of these populations had been subjected to monthly subnematicidal stress for over three years (stressed populations). Other populations had been released from the three years of subnematicidal stress for two years before being tested (unstressed populations). Diverse altered behaviors such as nonfumigant nematicide (NFN) resistance and increased susceptibility were observed. Results from subsequent *in vitro* bioassays with these populations closely paralleled the previous observations (Yamashita & Viglierchio, 1986*c*, 1987).

These earlier tests examined the long-term effects of stressing and unstressing. During preliminary experimentation, however, certain behaviors were observed in wild populations, which appeared to be influenced by a subnematicidal treatment applied five days before testing. A characterization of an immediate effect of NFN treatments on nematodes would provide an additional foundation for planning pest control stategies. These experiments were conducted to examine the short-term effects of selected chemical treatments on NFN tolerance in wild populations of *X. index* and *P. vulnus*.

Materials and methods

The tested species included wild populations (with no previous history of nematicide treatments) of *X. index* and *P. vulnus*. Stock populations of *X. index* were cultured on Carignan grapevines. The soil consisted of a sterilized mixture of equal parts loam and river sand. *Pratylenchus vulnus* was cultured on Kentucky Wonder bean plants grown in a sterilized mixture of equal parts white sand and river sand. All cultures were maintained in four liter pots and watered daily with half strength Hoagland's nutrient solution.

Experiments were conducted as follows. A tested stock culture pot was left unwatered for fourteen hours. Then, a subnematicidal concentration of either carbofuran, oxamyl or phenamiphos was applied. The concentrations used included the following : carbofuran - 0.004 mM; oxamyl - 0.006 mM; phenamiphos -

0.0024 mM. This application was sufficient to drench the pot to excess run-off. The treated pot was left unwatered for 24 hours before normal watering was resumed. At various days following NFN treatment (induction treatment) root and soil cores were removed from the test pot. Nematodes were extracted and examined for NFN tolerance through an *in vitro* bioassay as outlined in previous studies (Yamashita & Viglierchio, 1986*c*, 1987). An *in vitro* test was also conducted with the nematodes from the same culture pot before induction treatment. The response of these nematodes provided a base from which to gauge the short-term effects of this NFN treatment.

Results

Xiphinema index

Carbofuran induction treatment (CIT)

On day 30 following carbofuran induction treatment (CIT) the nematodes appeared to have declined in vigor (control treatment; Tab. 1). However, the CIT seemed to have conferred a degree of tolerance to all three nonfumigant nematicides (NFN). This induced tolerance was manifested as early as the fifth day following CIT. It appeared to be maintained through the fifteenth day before declining on days 30 and 45. The highest

only 42 % survived this 0.60 mM oxamyl treatment.

On day 45 the nematodes appeared to respond normally to all carbofuran concentrations inasmuch as the percent survival paralleled that observed before CIT. This pattern was not observed with all oxamyl concentrations nor at the lowest concentration of phenamiphos. Here, the percent survival, although appearing to decline from the former three assessment dates, remained significantly above the values recorded before CIT. On day 45 nematodes seemed to respond normally to 0.096 mM phenamiphos (36 % on day 45 *vs* 39 % before CIT). When exposed to 0.160 mM phenamiphos, however, there was a significant reduction in tolerance noticed on days 30 (16 % *vs* 23 % before CIT) and 45 (14 % *vs* 23 % before CIT).

Oxamyl induction treatment (OxIT)

The oxamyl induction treatment (OxIT) appeared to have no effect on the survival of nematode in the control treatment (Tab. 2). Increased tolerance, however, was expressed when the nematodes were exposed to the three nematicides. As with the CIT, this effect was seen as early as the fifth day following OxIT. In general the increased tolerance reached a peak at day five, maintained through day fifteen and began to decline on day 30. Yet, even on day 45 the nematodes displayed increased tolerance to all concentrations of carbofuran. The response to the two lower concentrations of oxamyl (79 % at 0.06 mM; 77 % at 0.30 mM) and to 0.032 mM and 0.160 mM phenamiphos (80 % at 0.032 mM; 4 % at 0.160 mM) seemed normal on day 45. However, the nematodes appeared to have developed an increased sensitivity to 0.60 mM oxamyl (39 % on day 45 *vs* 52 % before OxIT) and 0.096 mM phenamiphos (25 % on day 45 *vs* 46 % before OxIT).

There is an interesting pattern in the carbofuran and oxamyl treatments of the CIT and OxIT experiments. When the nematodes received a CIT, they retained a higher tolerance to oxamyl even at day 45 (Tab. 1). When the nematodes received an OxIT, the higher tolerance at day 45 was not to oxamyl but to carbofuran (Tab. 2). That is, there appeared to be a cross-tolerance expressed on day 45. The low percent of survival to 0.60 mM carbofuran expressed on day 30 (69 %) was apparently specific to that particular time period and conforms to no trend.

Table 2

Oxamyl induction of nematicide tolerance in a wild population or *Xiphinema index* : percent survival following a 24 hour exposure to three nematicides

Nematicide treatment	*Before induction treatment*	*Days following induction treatment*			
		day 5	*day 15*	*day 30*	*day 45*
Control	86 *DEF*	95 *ABC*	88 *DE*	90 *BCD*	85 *DEF*
Carbofuran :					
0.02 mM	78 *G*	97 *A*	88 *DE*	89 *CD*	87 *DEF*
0.20 mM	66 *H*	96 *AB*	86 *DEF*	85 *DEF*	81 *FG*
0.60 mM	58 *I*	90 *BCD*	86 *DEF*	69 *H*	82 *EFG*
Control	86 *cde*	95 *a*	88 *bcd*	90 *abcd*	85 *cdef*
Oxamyl :					
0.06 mM	79 *fg*	96 *a*	87 *bcde*	86 *cde*	79 *fg*
0.30 mM	70 *h*	93 *ab*	91 *abc*	79 *fg*	77 *g*
0.60 mM	52 *i*	84 *def*	81 *efg*	46 *i*	39 *j*
Control	86 $\beta\gamma$	95 α	88 β	90 $\alpha\beta$	85 $\beta\gamma$
Phenamiphos :					
0.032 mM	77 δ	96 α	87 β	86 $\beta\gamma$	80 $\gamma\delta$
0.096 mM	46 ζ	37 η	74 δ	59 ε	25 θ
0.160 mM	1 κ	4 κ	11 ι	17 ι	4 κ

The stock culture pot was treated with 0.006 mM oxamyl (induction treatment). Soil and root cores were removed 5, 15, 30 and 45 days following induction treatment. Extracted nematodes were exposed to three concentrations each of carbofuran, oxamyl and phenamiphos for 24 hours and then evaluated for active *vs* inactive using a touch-response method. Numbers represent the means of five replications. Means not followed by a common letter are significantly different at an α level of 5 % or less.

Phenamiphos induction treatment (PhIT)

On day fifteen the nematodes demonstrated a reduced ability to survive the induction treatment (79 % on day 15 *vs* 100 % before PhIT; Tab. 3). The culture of *X. index* used in this PhIT test was able to withstand the three nematicides better than the populations used in the CIT and OxIT (compare survival before induction treatments; Tab. 1, Tab. 2, Tab. 3). Variations between stock cultures within a population are common and the appropriate comparisons for induction effects should be made within the bounds of any one treatment.

Unlike responses from the CIT and OxIT, nematodes receiving a PhIT did not demonstrate consistent increases in tolerance to all nematicide concentrations. For example, on day 30 all concentrations of oxamyl and phenamiphos and the 0.60 mM exposure of carbofuran resulted in a significant reduction in survival percentages (compared to treatments of the wild-type population before induction). The more typical patterns of increased nematicide tolerance with time after induction were observed at 0.60 mM carbofuran, 0.60 mM oxamyl and 0.096 mM phenamiphos. At these concentrations, the nematodes displayed increased tolerance even on day 45. An alternating between a reduced or increased tolerance with time after induction, however, appeared to be common in these PhIT trials, and this effect can be seen in each of the three previously mentioned examples (0.60 mM carbofuran; 0.60 mM oxamyl; 0.096 mM phenamiphos). This type of effect was also seen in the OxIT trials (0.60 mM carbofuran). A consistent decrease in tolerance was demonstrated at only one nematicide exposure (0.160 mM phenamiphos). This consistent reduction in tolerance appeared to be unique to the PhIT as such an effect was not observed in the CIT or OxIT trials.

PRATYLENCHUS VULNUS

Carbofuran induction treatment (CIT)

The CIT appeared to increase tolerance to all three nematicides (Tab. 4). In most cases increased tolerance was evident at day 15 and was seen to improve on day 45. At 0.40 mM oxamyl, the percent survival was reduced on day 15 before increasing dramatically on day 45. This alternation between reduced and increased tolerance was also observed in phenamiphos induction trials with *X. index* (Tab. 3).

Table 3

Phenamiphos induction of nematicide tolerance in a wild population of *Xiphinema index* : percent survival following a 24 hour exposure to three nematicides

Nematicide treatment	*Before induction treatment*	*Days following induction treatment*			
		day 5	*day 15*	*day 30*	*day 45*
Control	100 *A*	94 *ABC*	79 *E*	93 *BCD*	100 *A*
Carbofuran :					
0.02 mM	100 *A*	94 *ABC*	87 *D*	95 *ABC*	100 *A*
0.20 mM	99 *AB*	96 *AB*	95 *ABC*	95 *ABC*	99 *AB*
0.60 mM	89 *CD*	97 *AB*	96 *AB*	60 *F*	98 *AB*
Control	100 *a*	94 *abcd*	79 *e*	93 *bcd*	100 *a*
Oxamyl :					
0.06 mM	100 *a*	98 *abc*	78 *e*	92 *cd*	99 *ab*
0.30 mM	97 *abc*	81 *e*	96 *abc*	48 *g*	96 *abc*
0.60 mM	80 *e*	67 *f*	89 *d*	24 *h*	95 *abcd*
Control	100 α	94 $\alpha\beta\gamma$	79 ε	93 $\beta\gamma$	100 α
Phenamiphos :					
0.032 mM	98 $\alpha\beta$	98 $\alpha\beta$	86 δ	90 $\gamma\delta$	99 $\alpha\beta$
0.096 mM	49 θ	72 ζ	78 $\varepsilon\zeta$	28 ι	64 η
0.160 mM	31 ι	32 ι	28 ι	14 κ	19 κ

The stock culture pot was treated with 0.0024 mM phenamiphos (induction treatment). Soil and root cores were removed 5, 15, 30 and 45 days following induction treatment. Extracted nematodes were exposed to three concentrations each of carbofuran, oxamyl and phenamiphos for 24 hours and then evaluated for active *vs* inactive using a touch-response method. Numbers represent the means of five replications. Means not followed by a common letter are significantly different at an α level of 5 % or less.

Table 4

Carbofuran induction of nematicide tolerance in a wild population of *Pratylenchus vulnus* : percent survival following a 24 hour exposure to three nematicides

Nematicide treatment	*Before induction treatment*	*Days following induction treatment*	
		day 15	*day 45*
Control	99 *A*	100 *A*	100 *A*
Carbofuran :			
0.20 mM	69 *C*	95 *A*	99 *A*
0.40 mM	69 *C*	84 *B*	99 *A*
0.60 mM	55 *D*	82 *B*	100 *A*
Control	99 *a*	100 *a*	100 *a*
Oxamyl :			
0.04 mM	86 *cd*	98 *ab*	99 *ab*
0.20 mM	83 *de*	78 *e*	97 *ab*
0.40 mM	61 *f*	50 *g*	92 *bc*
Control	99 α	100 α	100 α
Phenamiphos :			
0.08 mM	39 ε	88 γ	99 α
0.20 mM	33 $\varepsilon\zeta$	69 δ	96 $\alpha\beta$
0.40 mM	31 ζ	39 ε	89 $\beta\gamma$

The stock culture pot was treated with 0.004 mM carbofuran (induction treatment). Soil and root cores were removed 15 and 45 days following induction treatment. Extracted nematodes were exposed to three concentrations each of carbofuran, oxamyl and phenamiphos for 24 hours and then evaluated for active *vs* inactive using a touch-response method. Numbers represent the means of five replications. Means not followed by a common letter are significantly different at an α level of 5 % or less.

Table 5

Oxamyl induction of nematicide tolerance in a wild population of *Pratylenchus vulnus* : percent survival following a 24 hour exposure to three nematicides

Nematicide treatment	*Before induction treatment*	*Days following induction treatment*	
		day 15	*day 45*
Control	99 *AB*	100 *A*	99 *AB*
Carbofuran :			
0.20 mM	56 *D*	95 *AB*	98 *AB*
0.40 mM	55 *D*	80 *C*	98 *AB*
0.60 mM	62 *D*	92 B	94 *AB*
Control	99 *a*	100 *a*	99 *a*
Oxamyl :			
0.04 mM	76 *b*	99 *a*	99 *a*
0.20 mM	58 *c*	95 *a*	96 *a*
0.40 mM	24 *d*	76 *b*	82 *b*
Control	99 α	100 α	99 α
Phenamiphos :			
0.08 mM	49 δ	97 α	99 α
0.20 mM	29 ε	94 α	96 α
0.40 mM	14 ζ	70 γ	84 β

The stock culture pot was treated with 0.006 mM oxamyl (induction treatment). Soil and root cores were removed 15 and 45 days following induction treatment. Extracted nematodes were exposed to three concentrations each of carbofuran, oxamyl and phenamiphos for 24 hours and then evaluated for active *vs* inactive using a touch-response method. Numbers represent the means of five replications. Means not followed by a common letter are significantly different at an α level of 5 % or less.

Oxamyl induction treatment (OxIT)

The effects of the OxIT are quite dramatic (Tab. 5). Increased tolerance to all three nematicides was demonstrated on day 15 and remained unchanged on day 45. In only one instance (0.40 mM phenamiphos) was the tolerance observed to increase from day 15 to day 45 (70 % on day 15 *vs* 84 % on day 45). An unusual increase in survival percentage was expressed in the transition from 0.40 mM to 0.60 mM carbofuran (80 % at 0.40 mM *vs* 92 % at 0.60 mM). Although not significantly different, this effect was observed in the population even before induction treatment (55 % at 0.40 mM *vs* 62 % at 0.60 mM). The dramatic effect of OxIT on increased tolerance was most evident when comparing the results at 0.40 mM phenamiphos (14 % before OxIT *vs* 84 % on day 45).

Phenamiphos induction treatment (PhIT)

The effects of the PhIT were similar to those observed in the CIT and OxIT trials (Tab. 6). The PhIT increased tolerance to all three nematicides. Furthermore, the effect observed on day 15 was also seen on day 45. As with the previous OxIT trial, an increase in tolerance between day 15 and day 45 occurred in the phenamiphos exposures (0.20 mM; 88 % on day 15 *vs* 99 % on day 45).

Discussion

The biochemical phenomenon of induction is well-known. An earlier paper, which discussed an operon model for regulation of protein synthesis, created much interest in this area of research (Jacob & Monod,

1961). Induction can occur through the activated synthesis of new enzymes or by the activation of existing proenzymes (Stryer, 1981). As an example of the latter, blood clotting in man is a protective response to trauma. A wound, for example, induces a series of biochemical transformations (or enzymatic cascade) which lead to eventual blood clotting (Jackson & Nemerson, 1980).

Table 6

Phenamiphos induction of nematicide tolerance in a wild population of *Pratylenchus vulnus* : percent survival following a 24 hour exposure to three nematicides

Nematicide treatment	*Before induction treatment*	*Days following induction treatment*	
		day 15	*day 45*

cases the interval between exposure and induction of significant amounts of detoxifying enzymes requires between two and three days (Terriere, 1984). However, in *S. eredania*, induction was evident as early as 30 mn following exposure (Brattsten, Wilkinson & Eisner, 1977). Whereas resistance tends to be more or less permanent, induction is temporary in nature. Furthermore, the inducing agents are selective, causing a greater production of certains forms of detoxifying enzymes than others (Terriere, 1984).

In comparison to *Aphelenchus avenae*, *Panagrellus redivivus* has demonstrated greater tolerance to toxic levels of phorate (Le Patourel & Wright, 1976). This was attributed in part to an higher rate of phorate metabolism by *P. redivivus*. A similar study with these two nematodes indicated that *P. redivivus* was more tolerant than *A. avenae* to toxic concentrations of aldicarb (Batterby, Le Patourel & Wright, 1977). This relative

harmful effect on phenamiphos-treated nematodes (control = 38 %; 0.02 mM carbofuran = 50 %; 0.06 mM oxamyl = 32 %; 0.032 mM phenamiphos = 1 %). The experiments were repeated with the nematodes placed under 7° for 72 hours. This resulted in a dramatic increase in the survival of all nematodes (control = 59 %; 0.02 mM carbofuran = 65 %; 0.06 mM oxamyl = 73 %; 0.032 mM phenamiphos = 86 %). Lastly, the experiments were repeated with all flasks being aerated continuously for 72 hours. This treatment provided the highest survival percentages (control = 94 %; 0.02 mM carbofuran = 97 %; 0.06 mM oxamyl = 87 %; 0.032 mM phenamiphos = 92 %).

Based upon these additional test results, it appears that the protective effects of induction treatment may in part be related to the protective effects of quiescence. The observed antimicrobial effect, however, may be limited to the immediate *in vitro* conditions and is possibly unrelated to actual test pot situations experienced during induction trials. In other studies an extreme state of anoxybiosis (cryptobiosis) was induced in *A. avenae*. Anoxybiosis was induced by subjecting the nematodes to more than six days of anaerobic conditions (Cooper & Van Gundy, 1971). While in this cryptobiotic state, no measurable metabolic activity was detected in *A. avenae*. When the nematodes were returned to aerobic conditions following 60 days, however, more than 85 % of the nematodes recovered to normal activity. The protective effect of induction treatments may operate by lowering the overall metabolism of nematodes. While in a temporary state of reduced metabolic activity (induced quiescence) the nematodes may express an increased tolerance to chemical exposures.

Conclusive statements

Results from these test trials indicated that a pretreatment of nematodes with subnematicidal levels of carbofuran, oxamyl or phenamiphos can induce a short-term tolerance to subsequent nematicidal-level exposures. Because the experiments were terminated on day 45, it is difficult to estimate the length of this short-term protection. While further research is required to elucidate the mechanisms, the practical significance of short-term tolerance to agriculture is clear. This is especially important when realizing the changing trends from once-yearly to split and repeated applications of nonfumigant nematicides. Further research with induc-

previously stressed with carbofuran or oxamyl, were more sensitive to phenamiphos applications (Yamashita, Viglierchio & Schmitt, 1986). Also, a population of *P. vulnus*, previously stressed with carbofuran, displayed increased sensitivity to oxamyl.

In accord with the future goals of improved pest control strategies, further research into the areas of NFN induction may provide some answers for their efficient design.

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