

Induction of short-term tolerance to nonfumigant nematicides in stressed and unstressed populations of *Xiphinema index*

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SUMMARY

Populations of *Xiphinema index* were stressed with monthly subnematicidal doses of either carbofuran, oxamyl or phenamiphos for five years (stressed populations). At the end of the third year, one half of these cultures were released from nematicide stress (unstressed populations). These were cultured in the absence of monthly stressing for two years. Then, both stressed and unstressed populations were treated with a subnematicidal level of nonfumigant nematicide (NFN). At various days following this treatment, nematodes were assessed for their tolerance to high concentrations of NFN. Results from this and a previous test with a wild population suggested that the demonstrated increase in tolerance to NFN may be operating by two separate mechanisms. One system is induced by the subnematicidal treatment. It is transitory and seems to operate in all populations. The other mechanism appears to be permanent and does not require induction. This latter system parallels resistance and operates in only certain stressed and unstressed populations of *X. index*.

RÉSUMÉ

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

monthly subnematicidal stressing with either carbofuran (C-S-P), oxamyl (Ox-S-P) or phenamiphos (Ph-S-P) (stressed populations). The other group had been stressed with subnematicidal doses of nonfumigant nematicides (NFN) for three years. At the end of the third year, however, these cultures were released from monthly NFN stress (unstressed populations). When the current tests were conducted, these cultures had been released from carbofuran (C-U-P), oxamyl (Ox-U-P) and phenamiphos (Ph-U-P) stressing for two years.

The methods used for induction testing were the same as outlined in a previous study (Yamashita & Viglierchio, 1987*b*). A four liter stock culture pot of the tested population was treated with a subnematicidal concentration of its respective NFN (for example, C-S-P and C-U-P with carbofuran). The subnematicidal NFN concentrations were as follows : carbofuran — 0.004 mM; oxamyl — 0.006 mM; phenamiphos — 0.0024 mM. At various days following this subnematicidal treatment (induction treatment) root and soil cores were removed from the stock pot. Extracted nematodes were then tested for their tolerance to three concentrations each of carbofuran, oxamyl and phenamiphos through an *in vitro* bioassay as used in previous studies (Yamashita & Viglierchio, 1986*c*, 1987*a*). Results from *in vitro* testing of the nematodes immediately before subnematicidal application provided a comparison for observing the effects of this induction treatment. The stressed populations used in these trials had received their last monthly stress treatment 35 days before this *in vitro* assessment.

Analysis of all data was conducted following a logit transformation ($\ln [\text{number living} + 0.5 \text{ divided by number dead} + 0.5]$). Duncan's Multiple Range Test was used in comparing mean differences with an upper significance level of 5 %.

Results

XIPHINEMA INDEX (STRESSED POPULATIONS)

Carbofuran induction treatment (CIT) of the carbofuran-stressed population (C-S-P)

On day 30 following the carbofuran induction treatment (CIT), the nematodes showed signs of reduced activity (control, Tab. 1). This effect was also observed

Table 1

Carbofuran induction of nematicide tolerance in a carbofuran-stressed population of *Xiphinema index* : percent activity 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
Stressed Control	99 A	93 ABC	99 A	82 D	92 ABC
Carbofuran :					
0.02 mM	99 A	95 ABC	99 A	89 CD	93 ABC
0.20 mM	98 AB	93 ABC	96 ABC	93 ABC	93 ABC
0.60 mM	97 ABC	97 ABC	98 AB	90 C	91 BC
Stressed Control	99 a	93 ab	99 a	82 c	92 ab
Oxamyl :					
0.06 mM	99 a	92 ab	99 a	93 ab	93 ab
0.30 mM	97 ab	93 ab	98 ab	96 ab	94 ab
0.60 mM	75 cd	91 b	99 a	77 cd	73 d
Stressed Control	99 α	93 αβ	99 α	82 γ	92 αβ
Phenamiphos :					
0.032 mM	93 αβ	96 αβ	98 α	89 βγ	90 β
0.096 mM	39 ε	58 δ	30 ζ	54 δ	33 εζ
0.160 mM	3 η	7 η	9 η	9 η	9 η

The stock culture pot was treated with 0.004 mM carbofuran (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 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.

with a wild population of *X. index* (Yamashita & Viglierchio, 1987b). In this previously cited study CIT appeared to have increased tolerance to all concentrations of the three NFN. However, unlike the wild

with the wild population (Tab. 2). Whereas the wild population showed an immediate increase in tolerance on day 5, the Ox-S-P generally demonstrated reduced tolerance. This effect was transitory, however, as the

Phenamiphos induction treatment (PhIT) of the phenamiphos-stressed population (Ph-S-P)

The phenamiphos induction treatment (PhIT) appeared to have affected the viability of the Ph-S-P (stressed control treatment, days 15 and 30; Tab. 3). A similar effect was observed in an earlier test with the wild population (Yamashita & Viglierchio, 1987*b*). As with the C-S-P, PhIT seemed to have had little effect on the behavior of the Ph-S-P at the lowest exposures. However, an observed effect of PhIT was an alternating between increased and reduced tolerance. For example, there was a decrease in activity percentage on day 15, when the nematodes were exposed to 0.30 mM oxamyl (63 % *vs* 81 % before PhIT). Yet, on day 5 (93 %) and day 30 (92 %) the nematodes displayed increased tolerance. This alternating effect of PhIT was also observed in earlier tests with the wild population. A concentration-dependence of the PhIT alternating effect was most evident in the phenamiphos treatments. For example, at 0.032 mM phenamiphos, there were reductions in activity percentages on days 5, 15 and 30, whereas at 0.160 mM the nematodes demonstrated increased tolerance on days 5 and 15 (in comparison to pre-induction

survival). This trend of concentration-dependent variation was also seen in similar exposures of a wild population previously induced with phenamiphos.

XIPHINEMA INDEX (UNSTRESSED POPULATIONS)

Carbofuran induction treatment (CIT) of the carbofuran-unstressed population (C-U-P)

There were isolated indications of increased tolerance to 0.60 mM oxamyl on day 15 (100 %) and 0.160 mM phenamiphos on day 5 (32 %; Tab. 4). Compared to a previously tested wild population, however, the general response of this C-U-P to CIT was relatively neutral. This neutral response of the C-U-P to CIT was most evident at the two lower concentrations of carbofuran and oxamyl and at 0.032 mM phenamiphos. The general reaction of the C-S-P to CIT was also found to be indifferent (Tab. 1). There was a general pattern of response in the C-U-P, however, which set it apart from the C-S-P and wild population. This was the apparent induction of heightened sensitivity to the upper concentrations of all three NFN. For example, on days 5, 30 and 45 of the 0.60 mM carbofuran exposures, lower

Table 3

Phenamiphos induction of nematicide tolerance in a phenamiphos-stressed population of *Xiphinema index* : percent activity 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
Stressed Control	98 A	93 AB	91 B	91 B	95 AB
Carbofuran :					
0.02 mM	98 A	91 B	92 AB	82 C	92 AB
0.20 mM	92 AB	89 B	77 C	92 AB	90 B
0.60 mM	93 AB	91 B	89 B	94 AB	92 AB
Stressed Control	98 a	93 abcd	91 abcd	91 abcd	95 abc
Oxamyl :					
0.06 mM	96 ab	88 cde	89 bcd	92 abcd	93 abcd
0.30 mM	81 e	93 abcd	63 fg	92 abcd	87 de
0.60 mM	43 h	64 fg	69 f	62 fg	58 g
Stressed Control	98 α	93 αβγ	91 αβγ	91 αβγ	95 αβ
Phenamiphos :					
0.032 mM	98 α	86 γ	86 γ	88 βγ	92 αβγ
0.096 mM	40 δε	38 ε	20 ζ	46 δ	41 δε
0.160 mM	1 τ	10 η	9 ηθ	5 ηθτ	3 θτ

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 carbofuran-unstressed population of *Xiphinema index*: percent activity 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
Unstressed Control	100 A	99 A	98 A	94 ABC	96 AB
Carbofuran :					
0.02 mM	98 A	99 A	99 A	98 A	97 A
0.20 mM	98 A	98 A	99 A	98 A	90 BCD
0.60 mM	97 A	85 D	96 AB	88 CD	87 D
Unstressed Control	100 a	98 ab	98 ab	94 abc	96 ab
Oxamyl :					
0.06 mM	99 ab	97 ab	99 ab	98 ab	96 ab
0.30 mM	92 bc	87 cd	98 ab	97 ab	87 cd
0.60 mM	84 d	71 e	100 a	88 cd	63 f
Unstressed Control	100 α	98 αβ	98 αβ	94 αβ	96 αβ
Phenamiphos :					
0.032 mM	92 β	99 αβ	98 αβ	95 αβ	94 αβ
0.096 mM	63 γδ	70 γ	19 ζ	62 δ	36 ε
0.160 mM	20 ζ	32 ε	9 η	3 η	2 η

The stock culture pot was treated with 0.004 mM carbofuran (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.

survival percentages were observed (85 %, 88 % and 87 % *vs* 97 % before induction). There was an isolated demonstration of increased sensitivity to 0.160 mM phenamiphos in the wild population (Yamashita & Viglierchio, 1987b). However, signs of heightened sensitivity were generally absent in the C-S-P.

Oxamyl induction treatment (OxIT) of the oxamyl-unstressed population (Ox-U-P)

The response of the Ox-U-P to OxIT closely paralleled that of the C-U-P to CIT (Tab. 5). There was relatively little change in the sensitivity of the Ox-U-P to all concentrations of carbofuran, the two lower exposures of oxamyl and to 0.032 mM phenamiphos. Oxamyl induction treatment, however, appeared to have induced a heightened sensitivity to 0.60 mM oxamyl and

sensitivity to nematicides. This effect was again observed with PhIT of the Ph-U-P (Tab. 6). At 0.60 mM carbofuran, for example, the nematodes displayed increased tolerance on day 5 (96 %), appeared normal on day 15 (72 %), and on days 30 and 45 were manifesting an heightened ability to withstand carbofuran exposures (93 % and 95 %). The overall response of the Ph-U-P to PhIT was in closer agreement with the wild population (Yamashita & Viglierchio, 1987b) than with the Ph-S-P (Tab. 3). For example, the signs of increased tolerance to the highest concentrations of carbofuran and oxamyl (Ph-U-P, Tab. 6) paralleled the response of the wild population. In addition, the reaction of the Ph-U-P to all phenamiphos exposures closely matched the activity of the wild population.

Table 5

Oxamyl induction of nematicide tolerance in an oxamyl-unstressed population of *Xiphinema index* : percent activity 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
Unstressed Control	99 A	88 C	99 A	93 ABC	94 ABC
Carbofuran :					
0.02 mM	99 A	94 ABC	98 A	93 ABC	94 ABC
0.20 mM	96 AB	93 ABC	96 AB	91 BC	94 ABC
0.60 mM	97 AB	96 AB	93 ABC	88 C	95 AB
Unstressed Control	99 a	88 bc	99 a	93 abc	94 abc
Oxamyl :					
0.06 mM	98 a	94 abc	98 a	93 abc	95 ab
0.30 mM	94 abc	94 abc	98 a	94 abc	87 c
0.60 mM	92 abc	92 abc	78 d	57 e	52 e
Unstressed Control	99 α	88 γ	99 α	93 αβγ	94 αβγ
Phenamiphos :					
0.032 mM	98 αβ	91 βγ	98 αβ	93 αβγ	94 αβγ
0.096 mM	73 δ	65 ε	76 δ	77 δ	36 ζ
0.160 mM	42 ζ	40 ζ	16 η	4 θ	5 θ

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 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.

subtle differences between stressed, unstressed and wild populations within the single species, *X. index*. While the unique combination of specific interacting factors may influence the expression of one particular type of response, a general characterization of wild, stressed and unstressed populations may be possible. The degree of response to induction treatments is generally greater in the wild population of *X. index* (Yamashita & Viglierchio, 1987b). This is not only exemplified through survival percentage comparisons before and after induction, but in the manifestation of these differences occurring even at the lowest nematicide exposures. Both the stressed and unstressed populations do not exhibit an induction effect until they are exposed to higher concentrations of nematicide. However, in most cases, the stressed and unstressed populations demonstrate a higher initial tolerance, even before induction treatments. This greater initial tolerance may be partly responsible for the seemingly indifferent response of stressed and unstressed populations to induction, expressed at the lower concentration exposures. More importantly, what this may imply is that the stressed and unstressed populations have an increased tolerance im-

printed in their system. However, it appears that increased tolerance must be induced in the wild population. The heightened survival percentages, resulting from induced tolerance, closely paralleled the initially high percentages in stressed and unstressed populations prior to induction. That is, the effects of induction may temporarily compensate for a lack of inherent resistance in the wild population.

Apparently, there may be two types of systems contributing to increased tolerance. One may be permanently imprinted into the physiology of the nematode and parallels the characteristic of resistance. The other system is temporary and may be induced by a subnematicidal exposure. It appears that the wild population can only utilize an inducible system of protection. Inherent tolerance in stressed and unstressed populations, however, may also be supplemented with an inducible mechanism. This latter effect, for example, is expressed with CIT of the C-S-P (0.60 mM oxamyl; Tab. 1).

Instituting an induction mechanism appears to have a draining effect on the energy systems of the nematode. This was exemplified with the lowered activity in control treatments following induction (for example, day

5, Tab. 2 and day 5, Tab. 5). Furthermore, in earlier trials stressed populations of *X. index* demonstrated lower reproductive potentials than the wild population (Yamashita, Viglierchio & Schmitt, 1986). When the nematodes were released from stress, however, not only was normal reproduction restored, but in one population nematode numbers were increased dramatically (Yamashita & Viglierchio, 1986a).

The energy draining effects of induction may partly explain how resistance was selected for in stressed populations. Nematodes placed under monthly stress treatments might be able to withstand treatments for some time by virtue of induced tolerance alone. However, the selective pressures would favor those nematodes with a more permanent or constitutive range of protective processes. This can be possible inasmuch as these nematodes would not have to draw upon a sudden redirection of energy to combat stress treatments. Rather, a more permanently imprinted tolerance would have allowed a so-called normal and viable functioning of its life processes.

Yet, in situations where a wild population is subjected to an occasional nematicide application, an induction mechanism may provide for nematode energy economy. This concept has been reviewed with respect to other organisms (Brattsten, 1979).

The PhIT effects of alternating increased and reduced tolerance is difficult to explain at this time. However, in earlier *in vitro* tests it appeared that phenamiphos affected a more complex range of physiological mechanisms in the nematode (Yamashita & Viglierchio, 1986c, 1987a). This was interpreted because of the greater degree of variation in nematode survival with changes in the concentration of phenamiphos. Sudden reductions in survival percentages appear to be related to the energy draining effects of induction. For example, the Ph-S-P demonstrated sudden reductions in tolerance to all three chemicals on day 15 (Tab. 3). This was the exact time period in which the Ph-S-P expressed reduced activity following induction (control; 98 % before induction vs 91 % on day 15).

Table 6

Phenamiphos induction of nematicide tolerance in a phenamiphos-unstressed population of *Xiphinema index* : percent activity 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
Unstressed Control	99 A	91 BC	88 C	88 C	98 A
Carbofuran :					
0.02 mM	98 A	94 ABC	91 BC	90 BC	96 AB
0.20 mM	98 A	95 AB	81 D	91 BC	98 A
0.60 mM	66 E	96 AB	72 E	93 ABC	95 AB
Unstressed Control	99 a	91 bcd	88 bcd	88 bcd	98 a
Oxamyl :					
0.06 mM	98 a	92 abcd	86 d	91 bcd	95 ab
0.30 mM	88 bcd	94 abc	78 e	92 abcd	87 cd
0.60 mM	61 g	93 abcd	62 fg	64 fg	68 f
Unstressed Control	99 α	91 $\beta\gamma$	88 γ	88 γ	98 $\alpha\beta$
Phenamiphos :					
0.032 mM	97 $\alpha\beta$	92 $\alpha\beta\gamma$	86 $\gamma\delta$	92 $\alpha\beta\gamma$	96 $\alpha\beta$
0.096 mM	53 ζ	57 ζ	50 ζ	80 δ	72 ϵ
0.160 mM	36 η	28 θ	16 τ	16 τ	21 τ

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.

Conclusive statements

Through *in vitro* bioassays, the nematicide tolerances of stressed and unstressed populations of *X. index* have been evaluated at various time periods following induction treatment. When results from these trials are compared to previous tests with a wild population, two mechanisms of increased tolerance may be inferred. One

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