

Field resistance to nonfumigant nematicides in *Xiphinema index* and *Meloidogyne incognita*

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SUMMARY

Populations of *Xiphinema index* and *Meloidogyne incognita* were sampled from three vineyard plots. Two had been treated with either carbofuran or phenamiphos. The third was an untreated control. In the fourth year all three plots were treated with carbofuran. Assessments in the first year of treatment indicated that carbofuran applications had reduced the *X. index* population to one half of the control levels. In the fourth year, however, the *X. index* population levels from the carbofuran and phenamiphos-treated vines were significantly higher than numbers taken from the control plot. Nematode numbers from the carbofuran-treated vines were more than three times the levels from the control plot. *In vitro* bioassays indicated that nematodes from the carbofuran and phenamiphos-treated plots had a higher tolerance to nonfumigant nematicides.

RÉSUMÉ

Résistance au champ de Xiphinema index et Meloidogyne incognita aux nématicides non fumigants

Des populations de *Xiphinema index* et *Meloidogyne incognita* ont été récoltées dans trois parcelles de vignes. Deux d'entre elles avaient été traitées avec du carbofuran ou du phenamiphos, la troisième constituant le témoin, non traité. La quatrième année les trois parcelles ont été traitées avec du carbofuran. L'évaluation faite au cours de la première année suivant le traitement indique que les applications de carbofuran ont réduit la population de *X. index* à la moitié de celle de la parcelle témoin. La quatrième année cependant les niveaux de population de *X. index* dans les parcelles traitées au carbofuran ou au phenamiphos étaient significativement plus élevés que ceux de la parcelle témoin. Le nombre de nématodes dans la parcelle traitée au carbofuran était plus de trois fois plus élevé que celui de la parcelle témoin. Des tests *in vitro* ont montré que les nématodes provenant des parcelles traitées au carbofuran ou au phenamiphos ont une tolérance plus élevée aux nématicides non fumigants.

Greenhouse and laboratory trials with *Xiphinema index*, *Meloidogyne incognita* and *Pratylenchus vulnus* have been conducted (Yamashita & Viglierchio, 1986a, 1986b, 1986c, 1987a; Yamashita, Viglierchio & Schmitt, 1986). One half of the populations had been stressed with monthly subnematicidal doses (stressed populations). The other half had undergone monthly subnematicidal treatments before being released from stress (unstressed). Comparisons with a wild population suggested that resistance to nonfumigant nematicides (NFN) had developed in certain stressed and unstressed populations.

The above tests were performed under controlled environmental conditions. A question arose, then, as to whether NFN resistance could develop under field situations. The following study addresses this question in the two species, *X. index* and *M. incognita*.

Materials and methods

A nine-year old Zinfandel vineyard (Lodi, California) was selected for this study. Dr. D. J. Raski had recently

completed nematicide testing in this vineyard. His study consisted of three plots (400 vines in each plot). One plot had a three-year history of carbofuran treatment. A second plot had been treated for three years with phenamiphos. The third represented an untreated control. Treated plots had received NFN applications once-yearly in mid February for three consecutive years (1982, 1983, 1984). The following rates and methods of application had been used: carbofuran — 11.4 kg a.i./ha, 10 % granular material broadcasted over 100 % of area and incorporated by cultivation; phenamiphos — 10.2 kg a.i./ha, emulsifiable concentrate shanked into 75 % of area. All plots were irrigated immediately following their treatments.

In early February 1985, populations of *X. index* were collected from carbofuran, phenamiphos and control plots. To increase the nematode numbers, the populations were inoculated onto two-month old Carignane grapevines. In late April, 1985 the nematodes were extracted using 833 μ m and 147 μ m sieves. The nematode suspension was further freed from debris using a Baermann funnel with three layers of cheesecloth.

Populations were assessed for the presence of resistance using an *in vitro* bioassay as outlined in a previous study (Yamashita & Viglierchio, 1987a).

After Dr. Raski had completed tests in 1984, the grower applied carbofuran to all plots in mid February, 1985 (emulsifiable concentrate sprayed over 50 % of area and irrigated shortly after; 4.5 kg a.i./ha). This provided an opportunity to inspect relative population levels from each plot. Dr. Raski's group had collected population data on *X. index* in 1982 and 1984. Their sampling was conducted as follows: from the 400 vines in each plot, eight were randomly selected for population assessments. Soil and root samples were collected with a shovel and nematodes were extracted using 833 µm and 147 µm sieves. Nematodes washed from the 147 µm sieve were counted directly.

In the current study every other vine was sampled with an Oakfield probe (200 vines sampled per plot). The soil and root cores from ten vines were combined to make one sample (thus, 20 samples per plot). Extraction and counting of *X. index* followed the same methods used by Dr. Raski's group. However, nematodes passing through the 147 µm sieve were caught on a 38 µm sieve. The collected nematodes were further freed from debris using a Baermann funnel with three layers of cheese-cloth. These were evaluated for *Meloidogyne*, *Pratylenchus*, *Criconebella*, *Paratylenchus*, free-living dorylaims and free-living nondorylaim species. Additional *X. index* found in these suspensions were omitted from final population counts. This population sampling was conducted in late May, 1985 (more than three months following the grower's carbofuran treatment). All population levels were expressed as the number of nematodes per 200 cm³ soil.

Additional soil and galled root samples were collected from control and carbofuran plots in late May, 1985. Following an inspection of ten perineal patterns from each plot sample (n = 20), the root-knot nematodes were tentatively identified as *Meloidogyne incognita*. Larvae were extracted under intermittent misting. These were assessed for NFN resistance using an *in vitro* bioassay (Yamashita & Viglierchio, 1987a).

All *in vitro* data were analyzed following a logit transformation (\ln [number live + 0.5 divided by number dead + 0.5]). Mean comparisons were evaluated by Duncan's Multiple Range Test with an upper significance level of 5 %.

Results

POPULATIONS LEVELS

In 1982 and 1984 population data was collected two months following chemical treatment. The carbofuran application appeared to have reduced nematode levels in 1982 (wild population = 102; carbofuran-treated =

56; Tab. 1). Because samples were not taken in 1983, it is difficult to estimate the populations that followed since 1982. However, the 1984 figures indicated only minor shifts in the nematode populations from 1982. Furthermore, the wild population levels remained significantly above the numbers recorded from the carbofuran plot.

Table 1
Field population levels of *Xiphinema index*
from a Zinfandel vineyard in Lodi, California

Field population	Year of population sampling		
	1982	1984	1985
Wild	102 a	75 b	29 e
Carbofuran-treated	56 c	50 cd	91 a
Phenamiphos-treated	not recorded	not recorded	43 d

Numbers represent the mean counts per 200 cm³ soil. In 1982 and 1984 soil and root samples were taken with a shovel from 8 randomly selected grapevines per treatment plot (400 vines per plot; data from a previous study by Dr. D. J. Raski). When Dr. Raski completed his study in 1984, the grower applied carbofuran to all the plots in mid February, 1985 (emulsifiable concentrate sprayed over 50 % of area; 4.5 kg ai/ha). The senior author sampled every other vine (200 vines/plot) with an Oakfield probe in May, 1985. The cores from the ten vines were grouped to make one sample (thus, 20 samples per plot). Means not followed by a common letter are significantly different at an α level of 5 % or less.

The grower's carbofuran application in February of 1985 appeared to have significantly reduced nematode levels in the control plot (wild population; Tab. 1). Hence, it is interesting to note the sudden development of increased population levels in the carbofuran plot. In addition there were significantly higher numbers of nematodes recorded from the phenamiphos than the control plot. Results from a population level assessment of other nematode species in 1985 also supported the trends observed with *X. index*. Most dramatic was the significantly higher numbers of free-living nondorylaims (control = 33/200 cm³; phenamiphos = 41/200 cm³; carbofuran = 76/200 cm³) and free-living dorylaims (control = 18/200 cm³; phenamiphos = 16/200 cm³; carbofuran = 33/200 cm³) found in the carbofuran-treated rows. Low levels of *Meloidogyne* (2/200 cm³), *Pratylenchus* (2/200 cm³) and *Criconebella* (6/200 cm³) species were observed in samples taken from vines treated with carbofuran (1985). However, these species could not be detected from control and phenamiphos-treated plots.

Table 2

In vitro bioassays with carbofuran, oxamyl and phenamiphos : percent activity of three field populations of *Xiphinema index*

Field populations	Carbofuran Treatment			
	Control	0.02 mM	0.20 mM	0.60 mM
Wild	92 ABC	95 AB	79 E	41 F
Carbofuran	85 CDE	88 BCD	83 DE	85 CDE
Phenamiphos	95 AB	96 A	98 A	87 CD
	Oxamyl Treatment			
	Control	0.06 mM	0.30 mM	0.60 mM
Wild	92 ab	89 ab	67 ef	63 f
Carbofuran	85 bc	88 ab	78 cd	71 de
Phenamiphos	95 a	92 ab	78 cd	44 g
	Phenamiphos Treatment			
	Control	0.032 mM	0.096 mM	0.160 mM
Wild	92 αβ	69 γ	35 ε	2 η
Carbofuran	85 β	89 αβ	56 δ	14 ζ
Phenamiphos	95 α	87 β	10 ζ	1 η

Numbers represent the means of ten replications. Nematodes were exposed to each of the three nematicides for 24 hours and then evaluated for active *vs* inactive using a touch-response method. Means not followed by a common letter are significantly different at an α level of 5 % or less.

IN VITRO TESTS FOR RESISTANCE

Xiphinema index

Nematodes from both carbofuran and phenamiphos-treated plots demonstrated greater tolerance to carbofuran (Tab. 2). The response to oxamyl, however, was not as well defined. Nematodes from the carbofuran plot exhibited higher tolerance to 0.30 mM and 0.60 mM oxamyl. Those from the phenamiphos-treated vines displayed an ability to better withstand 0.30 mM but not 0.60 mM oxamyl. This lack of distinct differences in tolerance levels to oxamyl was also observed in an earlier study with *X. index* (Yamashita & Viglierchio, 1987a). Nematodes from the carbofuran plot demonstrated higher tolerance to all concentrations of phenamiphos. In a previous test, nematodes that had been stressed with carbofuran exhibited a higher tolerance only at the lowest concentration of phenamiphos (Yamashita & Viglierchio, 1987a). This latter behavior was observed in nematodes extracted from the phenamiphos plot. At 0.096 mM, however, these nematodes appeared to be more sensitive to phenamiphos.

Meloidogyne incognita

Nematodes from the carbofuran plot demonstrated a higher tolerance to all three nematicides (Tab. 3). This was best expressed at the highest concentrations. In previous *in vitro* studies with *M. incognita* populations that had been stressed with carbofuran also showed a similar pattern of increased tolerance to the three nematicides (Yamashita & Viglierchio, 1986c).

Table 3

In vitro bioassays with carbofuran, oxamyl and phenamiphos : percent survival of two field populations of *Meloidogyne incognita*

Field populations	Carbofuran Treatment			
	Control	0.20 mM	0.40 mM	0.60 mM
Wild	100 A	97 A	75 B	22 C
Carbofuran	100 A	100 A	99 A	72 B
	Oxamyl Treatment			
	Control	0.04 mM	0.20 mM	0.40 mM
Wild	100 a	90 b	62 c	5 f
Carbofuran	100 a	98 ab	53 d	32 e
	Phenamiphos Treatment			
	Control	0.08 mM	0.20 mM	0.40 mM
Wild	100 α	85 βγ	63 δ	23 ε
Carbofuran	100 α	100 α	90 β	79 γ

Numbers represent the means of ten replications. Nematodes were exposed to each of the three nematicides for 24 hours and then evaluated for active *vs* inactive using a touch-response method. Means not followed by a common letter are significantly different at an α level of 5 % or less.

Discussion

Development of nonfumigant nematicide (NFN) resistance under field situations would seem unlikely inasmuch as the required degree of selective pressures may be absent (Wright, 1981). This is especially so when considering the buffering capacity of the soil environment. Carbofuran, for example, appears to be degraded in the soil within a general span of three weeks (Caro *et al.*, 1973; Williams, Pepin & Brown, 1976). Other studies have indicated that leaching of carbofuran in the soil can be extensive (Felsot & Wilson, 1980). Further-

more, relationships between previous applications, microbial enrichment and rapid degradation of carbofuran have been observed (Felsot, Maddox & Bruce, 1981; Gorder, Dahm & Tollefson, 1982).

However, under field situations, populations of *Pratylenchus scribneri* demonstrated increased tolerance to nematicidal levels of carbofuran (Smolik, 1978). The corn fields, on which these tests were conducted, had been treated for five consecutive years with carbofuran. Applications of phenamiphos or oxamyl over a three-year period were reported to have had little effect on vineyard populations of *Xiphinema index* and *X. pachtaicum* (Roca *et al.*, 1980). Populations of *Paratylenchus hamatus*, infesting greenhouse rose beds, also exhibited increased tolerance to aldicarb (MacDonald, 1976). The nematodes had been previously exposed to aldicarb through periodic control treatments (one and a half years duration). Furthermore, in spite of more than five consecutive years of phenamiphos control applications, it appears that nematode pests of bananas continued to be a major disease problem (Schmitt, pers. comm.).

Populations of *X. index*, which were stressed with subnematicidal levels of NFN for ever three years, indicated signs of resistance (Yamashita, Viglierchio & Schmitt, 1986). For example, populations that had been stressed with carbofuran showed signs of resistance to carbofuran, oxamyl and phenamiphos. Furthermore, a nematicidal-level application of carbofuran appeared to have a stimulatory effect on this population (control = 570 *vs* carbofuran treatment = 800). In this same study a *X. index* population, that had been previously stressed with phenamiphos, developed larger populations following a carbofuran and subnematicidal dose of phenamiphos (control = 1 214; carbofuran treatment = 2 438; phenamiphos treatment = 2 752). A stimulatory resurgence of populations, following insecticide treatment, had been observed in insects for some time (Ripper, 1956). Perhaps, factors such as these may help explain the significantly larger nematode populations found in the carbofuran and phenamiphos-treated vineyard plots (Tab. 1).

However unexpected, the results suggest that sufficient selective pressures developed under these field situations. The vineyard from which the test nematodes were taken was treated once-yearly for four consecutive years. While maintaining NFN-stressed cultures of *X. index*, *M. incognita* and *P. vulnus*, casual population level inspections were made every three to four months. In the order of lowest to highest population levels the consistent findings for each culture were as follows: *X. index* - 1. carbofuran 2. oxamyl 3. phenamiphos; *M. incognita* - 1. carbofuran 2. oxamyl 3. phenamiphos; *P. vulnus* - 1. phenamiphos 2. oxamyl 3. carbofuran. The observations with *X. index* and *M. incognita* seemed unusual inasmuch as *in vitro* tests had indicated that both species were least affected by carbofuran (Yamashita & Viglierchio, 1986c, 1987a). In fact *X. index* was

found to be most sensitive to phenamiphos. During initial greenhouse studies, a series of tests were designed from which the factor of microbial degradation could possibly be detected (Yamashita, Viglierchio & Schmitt, 1986). When nematodes were inoculated onto test plants, nematode-free leachings from respective stock culture pots were added. When the population levels were evaluated following nematicide treatments, some interesting results were observed. The effects of enhanced population reductions or enhanced protection from nematicidal doses varied with the specific nematode-nematicide interaction. It suggested that specific breakdown products of the parent nematicide (for example, sulfoxide or sulfone, etc.) were more or less toxic to particular nematode species and strains. In view of these factors and the results from the stock population levels, then, it appears that these particular NFN are capable of persisting in some form in the soil. Relationships found between relative water solubility and nematicide persistence may also be a consideration here (Hafez, Raski & Lear, 1981). The persistence may not necessarily be limited to the soil environment, however, as a residue study indicated that aldicarb could persist in grapevines for several months (Hafez & Raski, 1981).

Furthermore, recent trials with wild populations of *X. index* and *P. vulnus* indicate that a subnematicidal exposure can induce short-term tolerance to subsequent nematicidal-level doses (Yamashita & Viglierchio, 1987b). This mechanism of adaptation should not be looked upon as unusual to the species when realizing that nematodes have demonstrated a number of adaptive behaviors. For example, the directional movements of *Ditylenchus dipsaci* in temperature gradients is dictated by the temperature to which the nematode was previously exposed (Croll, 1967). Moreover, exposing *Caenorhabditis elegans* to elevated but tolerable temperatures induced the synthesis of eight different polypeptides (Snutch & Baillie, 1983). More recently are the findings of transposable elements in *C. elegans* (Liao, Rosenzweig & Hirsch, 1983). These and related factors may prove to be additional mechanisms in the nematodes' arsenal of adaptive behaviors.

Conclusive statements

The infrequent reports of field resistance may be explained in part by the relatively recent shift to nonfumigant nematicides. However, the authors believe that other factors may be responsible. For one, resistance is oftentimes masked by opposing forces. Nematode selection for resistance may not necessarily evolve concomitant qualities of fitness for the environment. In extreme cases, for example, a nematode population may be able to withstand nematicidal exposures but will demonstrate a low reproductive potential. When field

assessments for resistance are based upon population levels alone, this latter condition could be easily misinterpreted as "no resistance". Nematodes also demonstrate subtle as well as distinct host preferences. When cultured on one particular host (host no. 1), for example, the wild-type strain will reach significantly higher populations levels than the resistant strain. In some cases a fourteen-fold difference has been observed. Here, the resistant strain can demonstrate higher tolerance to a nematicidal application and still appear to be lower in numbers than the wild strain. When the two different populations are cultured on another host (host no. 2), however, the resistant and wild strains may both attain equal but low population levels. A farm advisor sampling for nematodes, then, would not find ample cause for nematicide investments. By the time host no. 1 is replanted, the resistant strain may have reverted back to the wild-type. Previous tests with stressed, unstressed and wild-type nematode populations indicate that all of the above situations may occur (Yamashita & Viglierchio, 1986a, 1986b, 1986c, 1987a, 1987b, 1987c; Yamashita, Viglierchio & Schmitt, 1986).

The results from the current tests suggest that the development of NFN resistance under field conditions does not require a continuous monthly stressing regime. Apparently, in the vineyard used for these tests, sufficient selective pressures were created to favor resistance. These developments may vary with the specific host, nematode species and strain, nematicide, soil types and related factors. However, it has become apparent that the concept of resistance should be considered in designing future nematode control programs.

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