Responses of nematodes to nematicidal applications following extended exposures to subnematicidal stress

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Long-term exposures of plant-parasitic nematodes, *Xiphinema index*, *Meloidogyne incognita*, and *Pratylenchus vulnus* to subnematicidal stresses of carbofuran, oxamyl and phenamiphos can alter their responses to subsequent nematicidal treatments. Responses vary not only with the different nematicide used but with the different nematode species. Subnematicidal stressing with carbofuran and phenamiphos reduced reproductive potential in *X. index*, while phenamiphos stressing reduced reproductive potential in *M. incognita*. However, carbofuran stressing appeared to increase reproductive potential in *P. vulnus*. Cross-susceptibility was observed in carbofuran and oxamyl stressing, which made *X. index* more susceptible to phenamiphos, while carbofuran stressing increased the susceptibility of *P. vulnus* to oxamyl. Long-term subnematicidal stressing of all three nematodes with the three nematicides appeared to induce resistance and cross-resistance phenomena in the form of indifference and increased populations in response to nematicidal treatments. In some cases the behavioral response took the form of an apparent habituation. Soil leachings from carbofuran and phenamiphos stock cultures of *X. index* provided protection from corresponding nematicidal treatments, while addition of leachings from oxamyl and phenamiphos cultures of *M. incognita* assisted in decreasing populations following the respective nematicidal applications. The addition of oxamyl leachings also contributed in decreasing *X. index* population levels.

Résumé

*Réaction des nématodes lors de traitements nématicides consécutifs à une exposition à des doses sublétales de nématicides*

L'exposition prolongée des nématodes phytoparasites *Xiphinema index*, *Meloidogyne incognita* et *Pratylenchus vulnus* à des doses sublétales (ou « choc subnématicide ») des nématicides carbofuran, oxamyl et phenamiphos peut troubler leur réaction aux traitements nématicides ultérieurs. Ces réactions dépendent non seulement de la nature du nématicide utilisé, mais aussi de l'espèce de nématode considérée. Le « choc subnématicide » provoqué par le carbofuran et le phenamiphos réduit le potentiel reproducteur de *M. incognita*. En revanche, le carbofuran accroît ce même potentiel chez *P. vulnus*. Une sensibilité croisée a été observée dans le cas de chocs provoqués par le carbofuran et l'oxamyl : l'un et l'autre produits rendent *X. index* plus sensible au phenamiphos, tandis que le carbofuran augmente la sensibilité de *P. vulnus* à l'oxamyl. Les « chocs subnématicides » provoqués par les trois nématicides sur les trois nématodes en cause semblent induire des phénomènes de résistances directe et croisée se traduisant par un maintien ou un accroissement des populations lors des traitements nématicides consécutifs à de tels « chocs ». Dans certains cas, la réaction de comportement des nématodes prend l'aspect d'une accoutumance. Les percolats de sol dans lequel *X. index* avait été élevé et qui contenait des doses sublétales de carbofuran ou de phenamiphos fournissent une protection contre les traitements avec le produit nématicide correspondant. En revanche, l'addition des percolats provenant de sol contenant des doses sublétales d'oxamyl ou de phenamiphos, et dans lequel était élevé *M. incognita*, active la diminution de la population lors du traitement nématicide ultérieur correspondant. L'addition de percolats d'oxamyl participe également à la diminution des populations de *X. index*.

With increasing restrictions placed upon the use of soil fumigants, the role of nonfumigant nematicicides (NFN) is receiving greater concern (Raski, 1981). Most of these NFN, particularly the organophosphates and carbamates, are believed to act on the acetylcholinesterase and related nerve transmission centers of nematodes (Corbett, 1974). At recommended field doses, the bulk of these nematicicides appear to act by impairing motility, dispersion, orientation and attraction to hosts, resulting in reduced feeding, development and reproduction (Marban-Mendoza & Viglierchio, 1980a, 1980b, 1980c).

Pesticide resistance, tolerance and related phenomena in insects has been well documented (Georghiou, 1981; Council for Agricultural Science and Technology, 1983) and similar behaviors have been recorded for animal-parasitic nematodes and free-living bacterial feeders (Drudge et al., 1964; Douglas & Baker, 1968; Drudge, 1970; Brenner, 1974; Coles, 1977; Hall, Campbell & Richardson, 1978; Burger, 1979; Vlassoff & Kettle, 1980; Le Jambre, Martin & Jarret, 1982). The survival of nematodes exposed to concentrations of a nematicide, considered lethal, suggested the possibility for this phenomenon to occur with plant-parasitic species (Trujillo-Alvarado, 1956). A fungal feeding nematode, *Aphelenchus avenae*, was reported to have developed resistance to the alkyl halide, ethylene dibromide (Castro
& Thomason, 1971). However, following eighteen months' conditioning of Ditylenchus dipsaci with sublethal doses of oxamyl and phenamiphos, the nematode species did not display a marked resistance to these chemicals, except perhaps some tendency towards resistance with phenamiphos stressing (Bunt, 1975).

Varying exposure periods, the type of nematicide used and the nematode species treated may explain in part the different responses. Populations of Pratylenchus scribneri treated for five years with carbofuran and Paratylenchus hamatus treated for one and a half years with aldicarb exhibited a tolerance and/or resistance to subsequent applications of these nematicides (Mac Donald, 1976; Smolik, 1978). Further, applications of phenamiphos or oxamyl over a three year period for possible subnematicidal treatments of carbofuran, oxamyl and phenamiphos appeared to have had little effect on nematode populations (Roca et al., 1980). Nematicidal doses of aldicarb, once used to effectively control D. dipsaci on flower bulbs, have been reported to be ineffective (Hart, 1983).

Preliminary observations had indicated the possibility of various responses and developments in monthly stressed populations to nematicidal exposures (the term stressed being used here in reference to monthly applications of subnematicidal doses to stock nematode cultures). The aim of this study was to help express and to identify these various types of responses using three different nematodes and three different nonfumigant nematicides.

**Material and Methods**

Greenhouse stock cultures were maintained in four liter cans using the following soil mixtures and grape cultivars for each nematode species: *Xiphinema index* — loam:river sand (1:1), Carignane; *Meloidogyne incognita* — river sand:white sand (2:1), French Colombard; *Pratylenchus vulnus* — river sand:white sand (2:1), Thompson Seedless. All pots were watered through drip lines delivering approximately 800 ml of one half strength Hoagland’s solution per day. The greenhouse temperature was maintained throughout the season at approximately 25°C.

In October of 1979 treatment of stock cultures were initiated with one liter of a subnematicidal concentration of each NPN. These concentrations were estimated by calculating a ten-fold dilution of the recommended field doses. Drip line valves were shut and the pots were allowed to dry for fourteen hours before each culture pot was treated with one liter of the respective chemical, sufficient to drench the excess run-off. The drip line valves were turned on following 24 hours of exposure. The technical grade nematicides included the following: 1) carbofuran (2, 3 - dihydro - 2, 2 - dimethyl - 7 - benzo-furanyl methyl carbamate); 2) oxamyl (methyl-N, N'-di-methyl-N-(methyl-carbomoyl) oxyl][-1-thioxamamidate); 3) phenamiphos (ethyl 4 (methylthio)-m-tolyl isopropylphosphoramidate). Each nematode stock culture consisted of four pots treated with carbofuran, four pots with oxamyl, four pots with phenamiphos and four pots without treatment, making a total of 48 stock pots. Subnematicidal treatments were repeated on a monthly basis and stock population levels monitored every three to five months. Whenever population levels appeared to have increased, subnematicidal doses were increased in concentration. Figure 1 depicts a time scale and the course of subnematicidal treatments. On the thirty sixth month populations treated with oxamyl and carbofuran appeared to have decreased slightly and the subnematicidal treatments were returned to their original concentrations. All pots were periodically checked for pH deviations from neutrality using leachings from 500 ml of applied half strength Hoagland’s solution.

After having been stressed with subnematicidal doses for about three years, the nematodes were examined for their possible development of behavioral changes. The tests were conducted in the following way:

*Xiphinema index*: Carignane grape seedlings were started from dormant two bud cuttings and planted in autoclaved six inch clay pots. After two month's growth, cylindrical holes were made in the soil on each side of the seedling trunk, the nematode suspension delivered, and the holes covered. Each pot was inoculated with an aliquant of 1000 mixed-stage nematodes extracted from the stock cultures. The population structure (percent contribution from various stages of the nematode) was recorded for each culture prior to inoculation.

The randomized complete block design consisted of five replications per treatment. Four sets (twenty pots/set) of potted grape seedlings were inoculated; three with nematodes stressed with carbofuran, oxamyl phenamiphos and the fourth with unstressed nematodes. The nematodes were allowed to establish for one week. Then, each set of five replicates received nematicidal treatments of carbofuran, oxamyl, phenamiphos and a control treatment of half strength Hoagland’s solution, making a total of sixteen treatments. A second series of pots were inoculated as above but at the time of inoculation also received 400 ml of leachings from the respective stock pots. During nematode extraction from stock cultures, the initial two liters of liquid from the washed and sieved soil was passed twice through a 38 μm sieve and allowed to settle overnight. These decanted leachings from carbofuran, oxamyl and phenamiphos stock pots were added along with their respective nematode inoculations. The object here was to see if any microbial or related factors were present in stock cultures, which may have been contributing to protecting the nematodes from applied nematicides or to their mortality. In order to observe the effects of subnematicidal doses on stressed populations, a third series of pots were inoculated with only stressed nematodes and later...
treated with their respective subnematicidal concentrations.

The method of applying nematicides was the same for all pots. Following inoculation, the nematodes were allowed to establish for one week. Then, drip emitters were shut and the pots allowed to dry for fourteen hours. Five hundred milliliters of each nematicide were used to drench to excess the respective pots at the following concentrations: 1) carbofuran - nematicidal (0.040 mM); subnematicidal (0.004 mM); 2) oxamyl - nematicidal (0.100 mM); subnematicidal (0.010 mM); 3) phenamiphos - nematicidal (0.016 mM); subnematicidal (0.0016 mM). The treatment was repeated after 24 hours and 48 hours. The entire application allowed 72 total hours of exposure to the nematicides, after which the drip emitters were turned on. Each emitter delivered approximately four evenly spaced applications totaling 700 mls of half strength Hoagland's solution per day.

All pots were harvested after two months. The soil from each pot was washed three times successively, each washing containing nematodes in suspension being sieved through 833 μm and 147 μm sieves. Nematodes caught on the 147 μm sieve were washed into flasks for later counting. The suspension passing through the 147 μm sieve was caught on a 43 μm sieve and the washings from these placed on Baerman funnel extraction for one week. Root weights, degree of galling and top appearance were recorded for each grapevine.

*Meloidogyne incognita*: French Colombard grape seedlings were used. The experimental design and methods were similar to those used for *X. index* except that four replications were used instead of five and 1 200 J2 nematodes aliquanted per pot instead of 1 000 for *X. index*. Nematicides were similarly applied but at the different concentrations: 1) carbofuran - nematicidal (0.020 mM); subnematicidal (0.002 mM); 2) oxamyl - nematicidal (0.050 mM); subnematicidal (0.005 mM); 3) phenamiphos - nematicidal (0.016 mM); subnematicidal (0.0016 mM). After two months, soil from each pot was washed three times as before with *X. index* and each washing was sieved through 246 μm and 38 μm sieves. Collections from the 38 μm sieves were extracted by Baerman funnel for one week. Following a grading of the tops and roots for vigor and the roots for galling, the roots were weighed, cut into 1.5 cm pieces and a 60 g aliquot was misted for ten days. Nematodes were collected every two days and stored in refrigerated flasks for later counting.
Pratylenchus vulnus: Thompson Seedless grape seedlings were prepared in the same way as were French Colombard and Carignane. The experimental design and methods were almost identical to those used for M. incognita except for inoculation with 500 mixed stage nematodes per pot, the recording of root lesions rather than galls, and the use of five replications. The population structure was recorded for the various cultures prior to inoculation (percent contribution from each nematode life stage).

All population data were analyzed following a Log, (X + 1) transformation. Statistical significance among all treatment means was gauged by Duncan’s Multiple Range Test. An a level of 5 % was used as the guideline for evaluation of significant differences.

Results

These initial greenhouse tests were evaluated under six major areas of concern:
1. Effects of monthly subnematicidal stressing on nematode reproduction.
2. Increased susceptibility of stressed populations to nematicidal applications.
3. Resistance development in stressed populations as evidenced by an indifferent response to nematicidal applications.
4. Resistance development in stressed populations as evidenced by their numbers being larger than the wild population following nematicidal treatment.
5. Resistance development in stressed populations as evidenced by an apparent habituation to subnematicidal applications.
6. The role of microbial-mediated and/or spontaneous nematicide breakdown.

To simplify the reading, four abbreviations will be used in this paper:
C-S-P = Carbofuran-Stressed Population
Ox-S-P = Oxamyl-Stressed Population
Ph-S-P = Phenamiphos-Stressed Population
W-P = Wild Population (unstressed control population)

Effects of Monthly Subnematicidal Stressing on Nematode Reproduction

The isolated effects of monthly stressing on reproduction can in part be evaluated by comparing nematode numbers from untreated wild and untreated stressed populations.

Pratylenchus vulnus: The carbofuran (570) and phenamiphos-stressed controls (1 214) were significantly lower than both the oxamyl-stressed (4 068) and wild population controls (5 334) (Tab. 1). In addition, the C-S-P control was significantly lower than the numbers from the Ph-S-P control.

Meloidogyne incognita: Although appearing to be different, there were no significant variations between the W-P (151 136), C-S-P (73 114) and Ox-S-P (57 280) controls (Tab. 2). However, the nematode number from the Ph-S-P control (14 689) was significantly below that of all three.

Pratylenchus vulnus: Nematode numbers from the C-S-P control (605) were significantly larger than the W-P control (76) (Tab. 3). Differences between stressed population controls were absent at the 5 % level.

Increased Susceptibility of Stressed Populations to Nematicidal Applications

Increased susceptibility can be inferred when treated stressed population numbers are found to be lower than:
1) nematicide treatment to the wild population
2) wild population control
3) stressed population control. Of the three comparisons, only the first two would require statistically significant differences. The most convenient method of observing this in the tables is to move down a chemical treatment column and locate a number lower than the last number in that column (the last number being the wild population response to the chemical). If the number is also lower than the W-P control and the appropriate stressed population control, increased susceptibility can be inferred.

Xiphinema index: A good example is found in Table 1. Moving down the phenamiphos treatment column it can be seen that the C-S-P number (320) is significantly lower than the treated W-P (4 966) and the W-P control (5 334). When looking across the table, it can also be seen that phenamiphos treatment of the C-S-P gives a number lower than the C-S-P control (570). The Ox-S-P reacts in a very similar manner to phenamiphos treatment. In this latter case the population level from phenamiphos treatment of the Ox-S-P (1 196) is significantly lower than the treated W-P (4 966), the W-P control (5 334) and the Ox-S-P control (4 068).

Meloidogyne incognita: Carbofuran treatment of the C-S-P (38 905) gives a number that is significantly lower than the treated W-P (110 154) near the 10 % level of significance. The treated C-S-P level is significantly lower than the treated W-P (4 966), the W-P control (5 334) and the Ox-S-P control (4 068).

Increased susceptibility of stressed populations can in part be evaluated by comparing nematode numbers from untreated wild and untreated stressed populations.

Xiphinema index: The carbofuran (570) and phenamiphos-stressed controls (1 214) were significantly lower than both the oxamyl-stressed (4 068) and wild population controls (5 334) (Tab. 1). In addition, the C-S-P control was significantly lower than the numbers from the Ph-S-P control.

Pratylenchus vulnus: Based on these results, it appears that carbofuran stressing of P. vulnus has increased its susceptibility to oxamyl treatment. The levels from the C-S-P (22) are significantly below the treated W-P (71), W-P control (76) and the C-S-P control (605).

Resistance Development in Stressed Populations as Evidenced by an Indifferent Response to Nematicidal Applications

When treatment to a W-P reduces nematode numbers significantly below the W-P control, a similar effect
Table 1
Mean nematode numbers from four populations of *Xiphinema index* following control, subnematicidal and nematicidal-level treatments

<table>
<thead>
<tr>
<th>X. index population</th>
<th>Carbofuran</th>
<th>Oxamyl</th>
<th>Phenamiphos</th>
<th>Control</th>
<th>Subnematicidal</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-S-P</td>
<td>800 efg</td>
<td>626 fgh</td>
<td>320 h</td>
<td>570 h</td>
<td>574 gh</td>
</tr>
<tr>
<td>Ox-S-P</td>
<td>3 212 ab</td>
<td>2 160 bcd</td>
<td>1 196 defg</td>
<td>4 068 ab</td>
<td>3 922 ab</td>
</tr>
<tr>
<td>Ph-S-P</td>
<td>2 438 bc</td>
<td>1 460 cde</td>
<td>1 256 cdef</td>
<td>1 214 cdef</td>
<td></td>
</tr>
<tr>
<td>Wild</td>
<td>900 efg</td>
<td>1 370 cde</td>
<td>4 966 a</td>
<td>5 334 a</td>
<td></td>
</tr>
</tbody>
</table>

Means not followed by a common letter are significantly different at an α level of 5% or less. C-S-P = Carbofuran-Stressed Population; Ox-S-P = Oxamyl-Stressed Population; Ph-S-P = Phenamiphos-Stressed Population; Wild = Unstressed Control Population.

Table 2
Mean nematode numbers from four populations of *Meloidogyne incognita* following control, subnematicidal and nematicidal-level treatments

<table>
<thead>
<tr>
<th>M. incognita population</th>
<th>Carbofuran</th>
<th>Oxamyl</th>
<th>Phenamiphos</th>
<th>Control</th>
<th>Subnematicidal</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-S-P</td>
<td>38 905 cdef</td>
<td>99 312 abc</td>
<td>39 355 cdef</td>
<td>73 114 abc</td>
<td>196 789 a</td>
</tr>
<tr>
<td>Ox-S-P</td>
<td>69 984 abde</td>
<td>56 105 bdef</td>
<td>52 240 bdef</td>
<td>57 280 bdef</td>
<td>125 314 abc</td>
</tr>
<tr>
<td>Ph-S-P</td>
<td>21 878 g</td>
<td>26 977 defg</td>
<td>15 171 g</td>
<td>14 689 g</td>
<td>20 370 fg</td>
</tr>
<tr>
<td>Wild</td>
<td>110 154 abc</td>
<td>48 753 cdef</td>
<td>22 387 defg</td>
<td>151 136 ab</td>
<td></td>
</tr>
</tbody>
</table>

Means not followed by a common letter are significantly different at an α level of 5% or less. C-S-P = Carbofuran-Stressed Population; Ox-S-P = Oxamyl-Stressed Population; Ph-S-P = Phenamiphos-Stressed Population; Wild = Unstressed Control Population.

Table 3
Mean nematode numbers from four populations of *Pratylenchus vulnus* following control, subnematicidal and nematicidal-level treatments

<table>
<thead>
<tr>
<th>P. vulnus population</th>
<th>Carbofuran</th>
<th>Oxamyl</th>
<th>Phenamiphos</th>
<th>Control</th>
<th>Subnematicidal</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-S-P</td>
<td>417 ab</td>
<td>22 g</td>
<td>0 g</td>
<td>605 a</td>
<td>508 a</td>
</tr>
<tr>
<td>Ox-S-P</td>
<td>431 ab</td>
<td>167 cde</td>
<td>95 bcd</td>
<td>436 abc</td>
<td>268 abed</td>
</tr>
<tr>
<td>Ph-S-P</td>
<td>454 ab</td>
<td>78 def</td>
<td>6 g</td>
<td>276 abc</td>
<td>319 abc</td>
</tr>
<tr>
<td>Wild</td>
<td>49 fg</td>
<td>71 ef</td>
<td>4 g</td>
<td>76 cde</td>
<td></td>
</tr>
</tbody>
</table>

Means not followed by a common letter are significantly different at an α level of 5% or less. C-S-P = Carbofuran-Stressed Population; Ox-S-P = Oxamyl-Stressed Population; Ph-S-P = Phenamiphos-Stressed Population; Wild = Unstressed Control Population.

might be expected in stressed populations. However, if a stressed population is not comparably reduced below a stressed population control, resistance to the nematicide may be suspected. This can be observed most easily in the tables by first moving across the wild population row. If a number is found to be significantly lower than the far right number (the W-P control), all other numbers in the column above this number can be compared to their respective stressed population controls. *Xiphinema index*: Moving across the wild population column (Tab. 1), it can be seen that both carbofuran (900) and oxamyl (1 370) treatment of the W-P have
Table 4
Effects of adding stock culture leachings to test pots

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Population Mean</th>
<th>(T + L) - T</th>
<th>Duncan's MRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xiphinema index</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-S-P + Leachings</td>
<td>1 896</td>
<td>+ 1 096</td>
<td>≈ 0.5 %</td>
</tr>
<tr>
<td>C-S-P</td>
<td>800</td>
<td>- 792</td>
<td>≈ 6.0 %</td>
</tr>
<tr>
<td>Ox-S-P + Leachings</td>
<td>1 368</td>
<td>+ 513</td>
<td>≈ 10.0 %</td>
</tr>
<tr>
<td>Ox-S-P</td>
<td>2 160</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ph-S-P + Leachings</td>
<td>1 769</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ph-S-P</td>
<td>1 256</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meloidogyne incognita</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-S-P + Leachings</td>
<td>37 151</td>
<td>- 14 738</td>
<td>NS</td>
</tr>
<tr>
<td>C-S-P</td>
<td>51 889</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ox-S-P + Leachings</td>
<td>8 314</td>
<td>- 59 871</td>
<td>≈ 4.0 %</td>
</tr>
<tr>
<td>Ox-S-P</td>
<td>68 185</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ph-S-P + Leachings</td>
<td>7 969</td>
<td>- 16 835</td>
<td>≈ 6.0 %</td>
</tr>
<tr>
<td>Ph-S-P</td>
<td>24 604</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pratylenchus vulnus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-S-P + Leachings</td>
<td>601</td>
<td>+ 184</td>
<td>NS</td>
</tr>
<tr>
<td>C-S-P</td>
<td>417</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ox-S-P + Leachings</td>
<td>140</td>
<td>- 27</td>
<td>NS</td>
</tr>
<tr>
<td>Ox-S-P</td>
<td>167</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ph-S-P + Leachings</td>
<td>32</td>
<td>+ 26</td>
<td>NS</td>
</tr>
<tr>
<td>Ph-S-P</td>
<td>6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

T + L = Nematicidal Treatment to Stressed Nematodes + Leachings.
NS = No Significant Difference.
C-S-P = Carbofuran-Stressed Population.
Ox-S-P = Oxamyl-Stressed Population.
Ph-S-P = Phenamiphos-Stressed Population.
Each population has been treated with its respective nematicide (e.g. C-S-P + L and C-S-P have been treated with carbofuran).

significantly reduced the levels below the W-P control (5 334). Carbofuran and oxamyl treatment of the stressed populations can now be evaluated against the respective stressed population controls. When making this comparison, it can be seen that all stressed population levels in each column are not significantly different from their respective controls. The absence of coinciding reductions of the stressed populations as was found in the treated W-P is what is referred to as indifference in this section.

Meloidogyne incognita: In the W-P row (Tab. 2) it can be seen that oxamyl (48 753) and phenamiphos (22 387) treatments have reduced the population numbers below the W-P control (151 136). However, when treated with oxamyl or phenamiphos, all stressed populations are not reduced below their respective controls. Earlier with X. index the stressed populations displayed indifference to carbofuran and oxamyl treatment. With M. incognita stressed populations show an indifferent response to oxamyl and phenamiphos treatment.

Pratylenchus vulnus: Again, looking across the W-P row, it can be seen that both carbofuran (49) and phenamiphos (4) treatment of the W-P caused a significant reduction below the W-P control (76) (Tab. 3). All stressed populations treated with carbofuran, however, show an indifferent response in that their population levels are not significantly reduced below their respective controls. A degree of indifference is also seen with phenamiphos treatment of the Ox-S-P. Its number (95) was not found to be significantly below the Ox-S-P control (436).

RESISTANCE DEVELOPMENT IN STRESSED POPULATIONS AS EVIDENCED BY THEIR NUMBERS BEING LARGER THAN THE WILD POPULATION FOLLOWING NEMATICIDAL TREATMENT

Xiphinema index: The most dramatic example of this type of effect can be seen with carbofuran treatment of the Ox-S-P and Ph-S-P (Tab. 1). Here, it can be seen that the population numbers from the Ox-S-P (3 212) and Ph-S-P (2 438) are significantly larger than the W-P (900).

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Meloidogyne incognita: With respect to the larger population effect, no significant differences were observed.

Pratylenchus vulnus: The larger population effect observations overlap the results from the indifference effect (Tab. 3). When treated with carbofuran, all stressed population levels were found to be significantly larger than the W-P. This is also seen with phenamiphos treatment of the Ox-S-P, that number being significantly larger than the W-P.

Resistance development in stressed populations as evidenced by an apparent habituation to subnematicidal applications

In these tests it was observed that a nematicidal-level application to a respective stressed population (e.g. carbofuran on C-S-P) resulted in either a reduction below the control or a form of indifferent response. Thus, when populations numbers are seen to significantly increase above the controls after treatment, a form of resistance can be inferred. This was observed with some subnematicidal treatments and the effect appeared to approximate that of habituation.

Xiphinema index: This effect is seen with phenamiphos treatment of the Ph-S-P. The nematicidal-level treatment gives a population level (1256) almost identical to that of the Ph-S-P control (1214). However, the subnematicidal application of phenamiphos to the Ph-S-P (2752) yields a population number more than twice that of the Ph-S-P control. While not meeting the minimum requirement of statistical tests used here, this population increase was found to be significant at the 8% level (Tab. 1).

Meloidogyne incognita: Carbofuran treatment of the C-S-P (38905) and its respective C-S-P control (73114) are not found to be significantly different (Tab. 2). Similarly, oxamyl treatment of the Ox-S-P (56105) is not found to be different from the Ox-S-P control (57280). However, subnematicidal carbofuran applied to the C-S-P (196789) and subnematicidal oxamyl applied to the Ox-S-P (125314) yield numbers more than twofold that of their respective control populations. These were found to be different at a significance level of 9%.

Pratylenchus vulnus: There were no subnematicidal effects observed here.

The role of microbial-mediated and or spontaneous nematicide breakdown

The presence of biological and/or chemical factors in stock cultures can be partly evaluated by adding leachings to test pots. Test pots with nematodes + leachings and nematodes alone are then treated with respective chemicals. That is, if the leachings are added from oxamyl stock cultures, for example, these test pots are treated with oxamyl. Significant increases or decreases in nematode population levels in these pots from levels in test pots without leachings may indicate the presence or absence of the above factors.

Xiphinema index: The differences in population means between test pots with and without leachings are listed in Table 4. The level of statistical significance is listed to the immediate right of a mean comparison. With X. index the addition of stock culture leachings to test pots at the time of nematode inoculation appears to have affected final population levels. While the effect is one of increased population levels with carbofuran (+1096) and phenamiphos (+513), addition of oxamyl stock culture leachings appears to have decreased the numbers (—792).

Meloidogyne incognita: Addition of leachings from the oxamyl and phenamiphos stock cultures contributed in decreasing mean population levels (—59871 with oxamyl; —16835 with phenamiphos). The addition of carbofuran stock culture leachings also assisted in decreasing population levels, but this decrease was not found to be statistically significant. With X. index the addition of carbofuran and phenamiphos leachings contribute towards increasing, while addition of oxamyl leachings appears to help decrease numbers following chemical treatment. With M. incognita this effect is one of contributing towards decreasing population levels with all leaching additions.

Pratylenchus vulnus: The effects of leachings were not found to be significant. However, the general pattern of an increase with carbofuran and phenamiphos and a decrease with oxamyl coincides with the X. index results.

Discussion

The lack of consistent patterns in response to the three nematicides are complicated by apparent differences between chemicals, nematode species and their interactions. Intensive laboratory investigations on LC50 ranges (Bunt, 1975) and of concentrations needed for behavioral modifications (Marban-Mendoza & Viglierchio, 1980a, 1980b, 1980c) helped to define equivalent concentration capabilities of various nematicides. Varied results from field tests may indicate, among other factors, the need for considering nematicidal interactions with a specific nematode species. When realizing differences between nematode species, one may anticipate variable responses, and this may be supported by observations with fungal feeders, bacterial feeders and/or animal parasites. For example, in one study three animal-parasitic species treated with the anthelmintic Haloxon were shown to have acetylcholinesterases (AChE's) with low inhibition rate constants and differed only quantitatively from other susceptible species. Another two species, however, possessed AChE's which reactivated spontaneously after inhibition and thus
differed qualitatively from the susceptible species (Hart & Lee, 1966). The ganglion stimulants levamisole and pyrantel are effective against Ascaris lumbricoides but have shown relatively poor activity against Trichuris and Enterobius species (Coles, 1977). In addition, Aphielenchus axenae has been shown to be more susceptible than Panagrellus redvillus to both phorate and aldicarb. Both a higher rate of uptake and slower metabolism of aldicarb appeared responsible, and with phorate, A. axenae not only metabolized this chemical ten times slower but produced phoratoxin, a potent inhibitor of AChE (Le Patourel & Wright, 1976; Batterby, Le Patourel & Wright, 1977).

The remainder of the discussion will follow the same divisions and major headings as used in the results section.

**Effects of Monthly Subnematicidal Stressing on Nematode Reproduction**

The low numbers from stressed population controls in X. index (carbofuran and phenamiphos) and M. incognita (phenamiphos) and the large population seen in the C-S-P control of P. vulnus may be true characteristics of the parent stressed populations. This may indicate their respective low or high reproductive capacities. There is supporting evidence for these types of observations from tests conducted on other organisms. For example, a three to four-fold increase in egg production capacity was observed in a benzimidazole-resistant strain of Haemonchus contortus (Vlassof & Kettle, 1980) and a 62% increase in population over controls was observed in P. vulnus exposed for twenty days in 0.001 mM carbofuran (Marban-Mendoza & Viglierchio, 1980c). This same phenomenon was observed in the insect, Drosophila melanogaster, following sublethal exposures of dieldrin and in Diabrotica virgifera following sublethal exposures to carbofuran (Ball & Su, 1979). "Flarebacks" or "resurgences" in population levels of over 50 species of phytophagous arthropods have been known to occur following applications of once effective pesticides (Ripper, 1956). However, in subnematicidal carbofuran exposures of Acrobeles nanus, the number of eggs per female was markedly reduced. In this study gravid females were unaffected and it was concluded that decreased egg production resulted from effects at oocyte development (Wasilewska, Oloffs & Webster, 1975). A decrease in egg production, hatch and larval development have been reported in other studies (Starr, Mai & Abawi, 1978; Greco & Thomason, 1980; Marban-Mendoza & Viglierchio, 1980c; Krishnaprasad & Krishnapa, 1981; Rodriguez-Kabana, King & Pope, 1981; Sakhuja, Inderjit & Sharma, 1981).

Nematodes can recover from nematicidal exposures following placement in water (Bunt, 1975; Marban-Mendoza & Viglierchio, 1980c), and the free exchange of water through the body wall of nematodes has been documented (Marks, Thomason & Castro, 1968; Viglierchio, 1974). The decreased population in X. index (carbofuran and phenamiphos) and M. incognita (phenamiphos) stressed population controls may reflect a possible result of nematicide retention within the body. If so, however, one would expect that subsequent application of the respective nematicidal dose would result in a population reduction below the level of the stressed population control. This latter effect is not observed. However, in these same stressed population controls, the numbers are lower than or equal to the population levels from chemical treatment of the wild population. With respect to this latter observation the effects of retention may remain a possibility. For example, the C-S-P (570) and Ph-S-P (1214) control levels of X. index (Tab. 1) are equal to and below the carbofuran (900) and phenamiphos (4966) treatment levels of the W-P.

The low populations in the P. vulnus tests were later found to be partly reflective of a relatively unsuitable host. Thompson Seedless grape seedlings were used in the experiment, but observations of stock populations from an ongoing side test revealed an almost two-fold difference in the more suitable host, French Colombard.

**Increased Susceptibility of Stressed Populations to Nematicidal Applications**

Carbofuran and oxamyl stressing of X. index appears to have made them more sensitive to phenamiphos. This effect is also seen in carbofuran stressing of P. vulnus. When this C-S-P of P. vulnus is treated with oxamyl (Tab. 3), their numbers (22) are significantly reduced below the C-S-P control (605). However, the same oxamyl treatment to the W-P or Ox-S-P appeared to have had no effect in reducing the population levels.

Alternating applications with different pesticides is widely practiced in agriculture, which may be done for protective countermeasures against the possible occurrence of resistant strains. However, the cross-susceptibility phenomenon observed here may partly account for its observed effectiveness.

**Stressing Effects on Resistance Development to Nematicides**

Nematicide resistance is herein partially defined by the authors both on a quantitative and qualitative basis. Indifference or the lack of observable population reductions and an increase in population levels following treatment are quantitative measures. Habituation or dependence may define one qualitative aspect of the former two. There is considerable overlap between indifference, increased population and habituation, but these are treated separately to assist in understanding the occurrence of resistance.

Revue Nématol., 9 (1) : 49-60 (1986)
Resistance development in stressed populations as evidenced by an indifferent response to nematicidal applications

As stated earlier in the results, if the W-P is reduced following nematicide treatment, a similar reduction should occur with the stressed population. When the stressed population is not comparably reduced following treatment, resistance can be suspected. The important aspect of this manifestation of resistance is that it can easily go undetected in the field. For example, in the X. index tests (Tab. 1), carbofuran treatment reduces the W-P well below the W-P control (900 vs 5334). Examination of the C-S-P, however, shows no significant reduction below the C-S-P control (800 vs 570). These test pots may be equated with a field situation. If the field was sampled for population levels following carbofuran treatments, the buildup of resistance would go undetected, as both numbers (800 from C-S-P and 900 from W-P) are similar and low. What's more, if a significance level of 10% were to be weighed here, treatment of the C-S-P with carbofuran increased that population above the C-S-P control (800 vs 570). An issue that can be raised is whether or not this stressed population has the capacity to parasitize a host as does the wild population. During the test evaluations, root galling indexes were taken for X. index and M. incognita.

Stressed populations were observed to cause just as much galling as did the wild populations. Although this aspect requires further investigation, equivalent galling indexes between wild and stressed populations gave an indication of unhampered pathogenicity in stressed populations.

Inherent genetic components for resistance, a mutation or mutations or immigration from neighboring populations must exist or occur for resistance to develop (Brown, 1971). Since an immigration factor is absent in these tests, genetic components for resistance and/or mutation may be factors here. More importantly, if effective resistance is to develop, it must involve the evolution of the ability to survive treatment and the evolution of fitness for reproduction, and these may be proceeding at different rates (McEnroe & Naegle, 1968).

Resistance development in stressed populations as evidenced by their numbers being larger than the wild population following nematicidal treatment

This manifestation of resistance can be more easily detected in the field. Carbofuran treatment of the Ox-S-P and Pa-S-P of X. index (3 212 and 2 438), for example, resulted in population levels far in excess of the W-P response to carbofuran (900). The same chemical treatment to all stressed populations in P. vulnus gave numbers significantly larger than from the W-P. Similarly, phenamiphos treatment of the Ox-S-P of P. vulnus (95) resulted in a population level significantly above the W-P (4). What is obvious in these examples is the presence of both direct and cross-resistance. The large population seen in these examples may indicate that the ability to survive treatment and the fitness for reproduction have evolved together.

Resistance development in stressed populations as evidenced by an apparent habituation to subnematicidal applications

Habituation and/or cross-habituation may be interpreted from the previous section on increased populations. However, in these cases, the occurrence or exact nature of this phenomenon is unknown at this time. Habituation may be evidenced by a stimulatory resurgence of populations as observed with insects (Ripper, 1956), may take the form of dependence for normal activity as seen in a nematode (Brenner, 1974) or may be subtly masked within an apparent indifferent response. Perhaps the resurgence and/or dependence aspect may be seen with subnematicidal phenamiphos in X. index or subnematicidal carbofuran and oxamyl in M. incognita. Populations from these treatments exceeded their respective stressed population controls. The more subtle effects, however, were especially evident in the section covering indifferent responses.

If the ultimate measure of habituation is in reproduction and population numbers, one may speculate that: 1) resurgences and/or one form of dependence occurs when an ability to survive treatment and fitness for reproduction have evolved together; 2) another form of dependence occurs when the ability to survive treatment evolves faster than fitness for reproduction; 3) subtle masking or indifference may occur when (a) the ability to survive treatment lags slightly behind the evolution of fitness for reproduction (b) fitness for reproduction lags slightly behind the ability to survive nematicidal treatment. The former case (a) may be envisioned when one considers that a certain percentage of treated nematodes may escape lethal exposures (especially in field conditions and in endoparasites) but by virtue of a high reproductive capacity may reestablish a comparable population to that of nematodes able to survive treatment. Secondly, under subnematicidal exposures, the former case (a) may be expressed more easily. These aspects are important when considering the increasing interest in the use of drip irrigation systems as a vehicle for repeated, low level applications of nonfumigant nematicides. Although significance levels of 8-9% were required, subnematicidal phenamiphos on X. index (Tab. 1) and subnematicidal carbofuran and oxamyl on M. incognita (Tab. 2) appeared to stimulate increased populations levels. Upon examining the effects of the nematicidal dose treatments of these same chemicals, however, it can be seen that population levels are lower than from subnematicidal treatments.
THE ROLE OF MICROBIAL-MEDIATED AND/OR SPONTANEOUS NEMATICIDE BREAKDOWN

Microbial degradation of pesticides is well documented (Kauffman & Edwards, 1982). Various studies indicate a definite role of microorganisms (Caro et al., 1973; Mathur, Hamilton & Vrain, 1980; Williams, Pepin & Brown, 1980). These not only suggest a positive relationship between previous applications and a microbial enrichment (Felsot, Maddox & Bruce, 1981) but indicate a five to seven day lag time prior to rapid degradation, strongly suggestive of the role played by microorganisms (Venkateswarlu, Siddarama Gowda & Sethunathan, 1977; Ahmad, Walgenback & Sutter, 1979). Lastly, it is common knowledge that high soil pH can accelerate hydrolysis of the active nematicidal components.

With the X. index experiment, soil leachings from carbofuran stock cultures provided protection to carbofuran treatments and a similar but lesser degree of protection was obtained from applying soil leachings from phenamiphos stock cultures. The opposite effect, however, was seen for oxamyl leachings with M. incognita, where addition of leachings from oxamyl and phenamiphos stock cultures appeared to have caused a reduction in population levels. Because the pH of stock and test cultures deviated little from neutrality, this effect may be considered negligible. If the responsible factor or factors were microbial in nature, one might expect consistency for each nematicide. Apparently, the effect is more complex and may be more accurately measurable over a longer period of exposure to treatments. Each test utilized a different grape variety and the interaction of organisms with a specific host may have some bearing. We do know that several related phenomena have surfaced from stock cultures. The opposite effect, protective pressures from subnematicidal exposures can result in populations with a lower or high fitness for reproduction; 2) select for and/or condition nematodes to be more sensitive to nematicidal doses; 3) yield resistant populations with indifference to nematicidal doses; 4) yield resistant populations that show higher reproductive fitness; 5) yields cross-susceptible and cross-resistant populations; 6) yield populations that show an habituation to nematicides; 7) the leachings from stock cultures contain a factor or factors which cause an increase or decrease in nematicide-treated stressed nematodes; 8) there are real differences between nematode species in their response to nematicides.

The implications to agriculture may be as profound as those experienced with insecticides, bactericides and fungicides. Awareness of possible outcomes can eventually lead to highly refined methods of detecting the presence of such a phenomenon as pesticide resistance in insects (Voss, 1980). An important aspect is the ability to predict what may occur when a particular class of pesticide is applied to a specific group of pests. It has been shown that when exposed to certain pesticides, closely-related insect species exhibit identical or similar AChE inhibition patterns and that species from different orders can be distinguished on the basis of their relative inhibition sensitivities (Voss, 1981). As an example, differences in electrophoretic esterase patterns have been used to separate relatively organophosphorous-insensitive from sensitive wild strains of Rhabditis species (Onishi, Oki & Fujita, 1981). Should such a parallelism exist in species of plant-parasitic nematodes, it may be possible to define parameters more clearly and thereby plan a more effective control program.

As restrictions upon chemicals increase, a trend has been to move away from general metabolic inhibitors to forms acting at specific sites. Further, as systemically-acting forms are sought, specific site inhibitors may become even more common. The use of chemical mixtures, representing different modes of action, has proved effective in insect (Brown, 1976) and in bacterial and fungal pathogen control programs (Greenway & Whatley, 1975; Delp, 1980). This aspect may gain further avenues of flexibility in a soil nematicide application when considering effects upon nematodes shown with fungicides (Trudgill, 1975; Reddy & Kumar Rao, 1975; Dibs, 1976; Rodriguez-Kabana & King, 1977), herbicides (Frey, 1976; Osman & Viglerchio, 1981; Schmitt & Corbin, 1981), adjuvants (Abu Elamayem et al., 1979) and fertilizers (Mojtahedi & Lownsbery, 1976). Hopefully, the results reported here will assist in understanding the complexity of chemical-nematode interactions not unlike those experienced with insects, bacteria and fungi.

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