Adaptive responses of *Heterodera schachtii* populations to nematicidal applications of nonfumigant nematicides after stressing with sublethal doses

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SUMMARY

Persistent stressing of *Heterodera schachtii* by subnematicidal concentrations of carbofuran, oxamyl, phenamiphos and aldicarb generated altered behavioral responses to subsequent nematicidal doses. Subnematicidal stressing with carbofuran and oxamyl reduced the reproductive capacity, whereas stressing with phenamiphos and aldicarb resulted in an apparent increase in reproductive capacity over the respective controls. Any subnematicidal treatment of carbofuran or oxamyl-stressed populations tended to increase population levels but similar treatments of phenamiphos or aldicarb-stressed populations reduced population levels respective to controls. Effectiveness depended upon the particular nematicide. Forms of resistance, cross-resistance or habituation were evident depending upon the stressed population and nematicide. The population increases resulting from nematicidal doses of phenamiphos and oxamyl were lower than the overall mean of all treatments and, with minor exceptions, appeared to be more effective in the general inihibition of increases in population than carbofuran or aldicarb.

RÉSUMÉ

*Réactions d'adaptation de populations d'*Heterodera schachtii *à des applications de nématicides non-fumigants après sensibilisation par des doses sublétales*

La sensibilisation persistante d'*Heterodera schachtii* obtenue par action de concentrations subnématicides de carbofuran, d'oxamyle, de phenamiphos ou d'aldicarbe modifie les réactions comportementales aux doses nématicides de ces produits appliquées ultérieurement. La sensibilisation subnématicide par le carbofuran et l'oxamyle réduit le pouvoir reproducteur tandis que celle causée par le phenamiphos et l'aldicarbe accroît celui-ci par rapport aux témoins respectifs. Tous les traitements subnématicides par le carbofuran ou l'oxamyle tendent à accroître le niveau des populations, alors que les traitements similaires par le phenamiphos ou l'aldicarbe diminuent ce niveau par rapport aux témoins. L'action produite dépend donc de la nature du nématicide. Des formes de résistance simple, de résistance croisée, ou de tolérance ont été mises en évidence qui sont fonction de la population sensibilisée et du nématicide. Les augmentations de populations provoquées par les doses nématicides de phenamiphos et d'oxamyle étaient inférieures à la moyenne générale de tous les traitements et, à de rares exceptions près, apparaissent plus efficaces envers une inhibition générale des accroissements de population que dans le cas du carbofuran ou de l'aldicarbe.

The sugar beet nematode, *Heterodera schachtii* Schmidt, has been recognized for over a century as a plant-parasitic species of particular importance to sugar beets. It was implicated as the major contributing factor to the collapse of the sugar beet industry of Europe in the late 1800s (Thorne, 1961). Although, upwards of a hundred hosts including cultivated and weed plants are known, the major economic impact occurs with cultivars of two genera, *Beta* and *Brassica.* Nematode control by means of plant breeding for nematode resistant cultivars, pursued for decades, has proved to be elusive. The primary means of nematode control continues to be crop rotation with susceptible cultivars grown one in three years for no or very light infestations and less frequently for heavier infestations. In an effort to grow beets more frequently, fumigant nematicides were tested and shown to be effective though usually uneconomical. Increasing restrictions for the use of fumigants have promoted additional interest in the use of second-generation nonfumigant nematicides (NFN) as alternatives. For economic considerations, research has often involved very low doses either in more frequent applications, or as controlled release granular nematicides (Boehm, 1986). Unfortunately, these are precisely those stressing conditions favoring selection of development of tolerance or resistance to NFN (National Research Council,

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1986). The existence of this phenomena in nematodes has been established from field observations for *Pratylenchus scribneri* (Smolik, 1978), *Xiphinema index* and *Meloigogyne incognita* (Yamashita & Viglierchio, 1987 *c*); from greenhouse trials for *Paratylenchus hamatus* (MacDonald, 1976), for *Pratylenchus vulnus* (Yamashita & Viglierchio, 1986 *a*), for *Criconemella xenoplax* (Yamashita, Viglierchio & Kuo, 1988), for *Xiphinema index* (Yamashita & Viglierchio, 1986*b*; 1987*a*, *b*, *c*) and *Heterodera schachtii* (Viglierchio & Brown, 1989). Though the behavioral modification effected by nonlethal NFN stress appears widespread, the specific responses are varied depending upon the nematode species, the particular nonfumigant nematicide and perhaps the host plant. The following study addresses the response of *Heterodera schachtii* to different NFN treatments following long-term, repeated exposures to low concentrations of four commonly used NFN.

Materials and Methods

Approximately 2 000 freshly hatched J2 of *H. schachtii* were inoculated onto 45-day-old sugar beet seedlings growing in 4-liter pots of a sterilized mixture of equal parts river sand and fine quartz sand and watered with half-Hoagland nutrient solution. These stock cultures were allowed to establish for two months before initiating monthly low doses of NFN as outlined in a previous study (Yamashita, Viglierchio & Schmitt, 1986). The NFN and their initial concentrations in the soil solution were carbofuran and oxamyl (0.002 mM) and phenamiphos and aldicarb (0.0002 mM). The populations were monitored periodically, and as their numbers increased, the monthly NFN doses were stepped up in concentration. At the time of testing, the populations were receiving the following monthly treatments : carbofuran and oxamyl (0.008 mM), phenamiphos and aldicarb (0.0012 mM). The stressed populations had received a monthly subnematicidal dose 30 days prior to their extraction for testing. The cysts, wet sieved from soil, were caught on a 246 μm sieve, further purified through sugar flotation, fragmented to release eggs and the resultant mixture placed on a modified Baermann funnel in the mister for hatching. Approximately, 2 000 freshly hatched nematodes were inoculated onto 45-day-old cabbage plants (cv. Copenhagen) grown in 12.5 cm diameter clay pots containing a 1:1 mixture of sterilized river and white quartz sand. The test was conducted with four replications using a completely randomized design. After allowing the populations to estaplish for one week, the test pots were drenched to excess on three successive days with the following NFN : carbofuran (C) and oxamyl (Ox) (subnematicidal = 0.008 mM; nematicidal = 0.008 mM), phenamiphos (P) and aldicarb (A) (subnematicidal = 0.0012 mM; nematicidal = 0.012 mM). The methods of treatment were as outlined in a previous study (Yamashita, Viglierchio & Schmitt, 1986).

After two months, cysts were collected from the pots as previously described, and then they were air dried and counted. Populations were analyzed following a $\log_{10}$ (cyst plus white female population) transformation. Statistical comparisons were conducted using Duncan's multiple range test with an upper significant level of 5 %.

Results

Although experimental results exhibited considerable variation, five principal categories emerged from previous trials with other nematodes which facilitated an interpretation of the data :

1. Effect of monthly subnematicidal stressing on nematode reproduction.
2. Increased susceptibility of stressed populations to NFN applications.
3. Resistance to NFN in stressed populations appearing as :
   a. An " indifference " response to chemical treatments.
   b. A larger population level following chemical treatments.
   c. An apparent habituation to chemical treatments.

For brevity : CTL = control (no chemical treatment); the subscripts signifies subnematicidal dose, e.g., C_s = subnematicidal carbofuran; the subscript n signifies nematicidal dose, e.g., C_n = carbofuran nematicidal dose (10 × C_s). Nematode populations are indicated thusly : W-P = wild population (no previous exposure to NFN); C-S-P = carbofuran-stressed population and similarly for Ox-S-P, P-S-P and A-S-P.

1. *Reproduction modifications as a consequence of NFN stressing treatments* are best shown in a comparison of population determinations from the CTL column (Tab. 1). A significantly lower number of *H. schachtii* females (cyst and white female) were recovered from carbofuran and oxamyl-stressed populations (C-S-P = 10 447, Ox-S-P = 9 840) in comparison to wild (W-P = 22 856) indicating a reduced reproductive potential. In contrast, phenamiphos and aldicarb-stressed populations (P-S-P = 34 198, A-S-P = 31 405) have increased reproductive capacity over W-P, albeit at a level of significance less than 10 %.

2. *Enhanced susceptibility in stressed populations* may be indicated, wherein a stressed population, after treatment, falls below a) W-P control, b) the respective stressed population control, c) the same NFN treatment to the W-P. No *H. schachtii* populations met all three conditions, although several met one or two.

Table 1
Responses of Various Populations of *Heterodera schachtii* to Nonfumigant Nematicides

	Nematicide treatments (Subnematicidal)				
Populations	*Control*	*Carbofuran*	*Oxamyl*	*Phenamiphos*	*Aldicarb*
Wild	22 856 *jklmnop*	18 197 *ghijklmnop*	11 749 *cdefghi*	8 995 *cde*	16 106 *defghijklmn*
Carbofuran	10 447 *cdefg*	16 520 *defghijklmno*	17 498 *fghijklmno*	20 277 *hijklmnop*	11 246 *cdefgh*
Oxamyl	9 840 *cdefg*	11 967 *cdefghij*	12 618 *cdefghijk*	14 894 *defghijklmn*	10 046 *cdefg*
Phenamiphos	34 198 *p*	28 054 *nop*	26 485 *mnop*	12 706 *cdefghijk*	25 704 *lmnop*
Aldicarb	31 405 *op*	21 677 *ijklmnop*	13 274 *cdefghijk*	24 099 *klmnop*	14 859 *defghijklmn*
	Nematicide treatments (Nematicidal)				
Populations	*Control*	*Carbofuran*	*Oxamyl*	*Phenamiphos*	*Aldicarb*
Wild	22 856 *jklmnop*	16 672 *efghijklmno*	9 661 *cdefg*	4 875 *ab*	4 446 *ab*
Carbofuran	10 447 *cdefg*	11 588 *cdefghi*	8 770 *cde*	4 864 *ab*	15 136 *defghijklmn*
Oxamyl	9 840 *cdefg*	8 670 *cd*	12 218 *cdefghij*	3 855 *a*	11 066 *cdefgh*
Phenamiphos	34 198 *p*	13 677 *cdefghijkl*	12 823 *cdefghijk*	9 268 *cdef*	14 421 *defghijklm*
Aldicarb	31 405 *op*	15 704 *defghijklmn*	7 396 *bc*	12 618 *cdefghijk*	20 464 *hijklmnop*

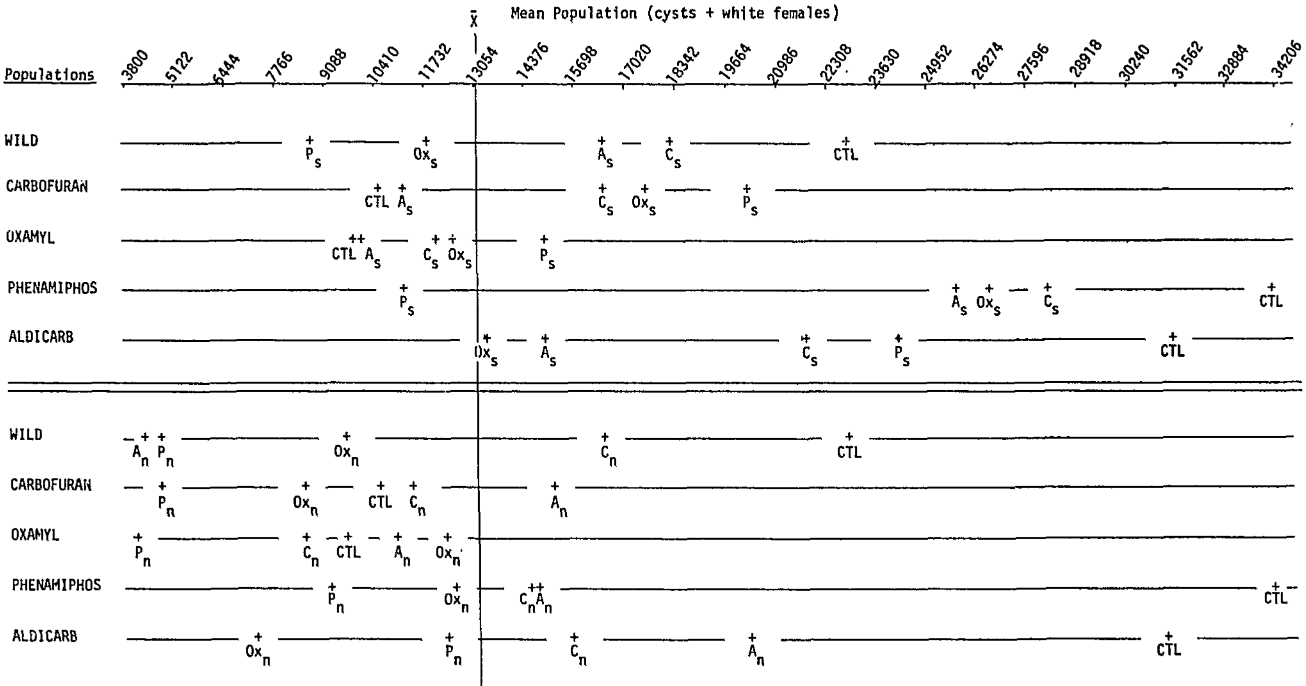
Numbers represent the mean population of cysts + white females. Those not followed by a common letter are different at a significance level of 5 % or less. Table 1A and 1B were analyzed together and the statistical designations apply to both.

3. a) A form of resistance to NFN in stressed populations can be suggested by an « *indifference* » response to chemical treatments. Should a chemical treatment which reduces the W-P below its respective control not generate a comparable reduction in its stressed population, then the absence of a comparable population reduction (indifference), suggests a degree of resistance to the NFN. No stressed population treated with any NFN generated a reduction from its control comparable to that generated with the W-P. Therefore, all stressed populations displayed a degree of tolerance to NFN at subnematicidal doses.

b) An additional form of resistance to NFN in stressed populations may be demonstrated by *larger population levels* following chemical treatments in comparison to like treated W-P. This form of resistance was demonstrated by several population-chemical combinations : Ox_s (P-S-P = 26 485 *vs* W-P = 11 749), P_s (A-S-P = 24 099 and C-S-P = 20 277 *vs* W-P = 8 995), P_n (A-S-P = 12 618, P-S-P = 9 268 *vs* W-P = 4 875), A_n (A-S-P = 20 464, P-S-P = 14 421, Ox-S-P- = 11 066, C-S-P = 15 136 *vs* W-P = 4 446). No effects of this nature were seen in C_s, A_s, C_n and Ox_n. A different perspective of the behavioral complexity is given by the observations of significant population reductions in the following cases : P-S-P (P_s = 12 706 *vs* CTL = 34 198), A-S-P (Ox_s = 13 274, A_s = 14 859 *vs* CT = 31 405), C-S-P (P_n = 4 864 *vs* CTL = 10 447), Ox-S-P (P_n = 3 855 *vs* CTL = 9 840), P-S-P (C_n = 13 677, Ox_n = 12 823, P_n = 9 268, A_n = 14 421 *vs* CTL = 34 198), and A-S-P (C_n = 15 704, Ox_n = 7 396, P_n = 12 618 *vs* CTL = 31 405). Except for C-S-P (P_s, A_n), Ox-S-P (C_n, A_n), P-S-P (Ox_s, P_n, A_n) and A-S-P (P_s, P_n, A_n), treatments are comparable to the corresponding treatment of W-P.

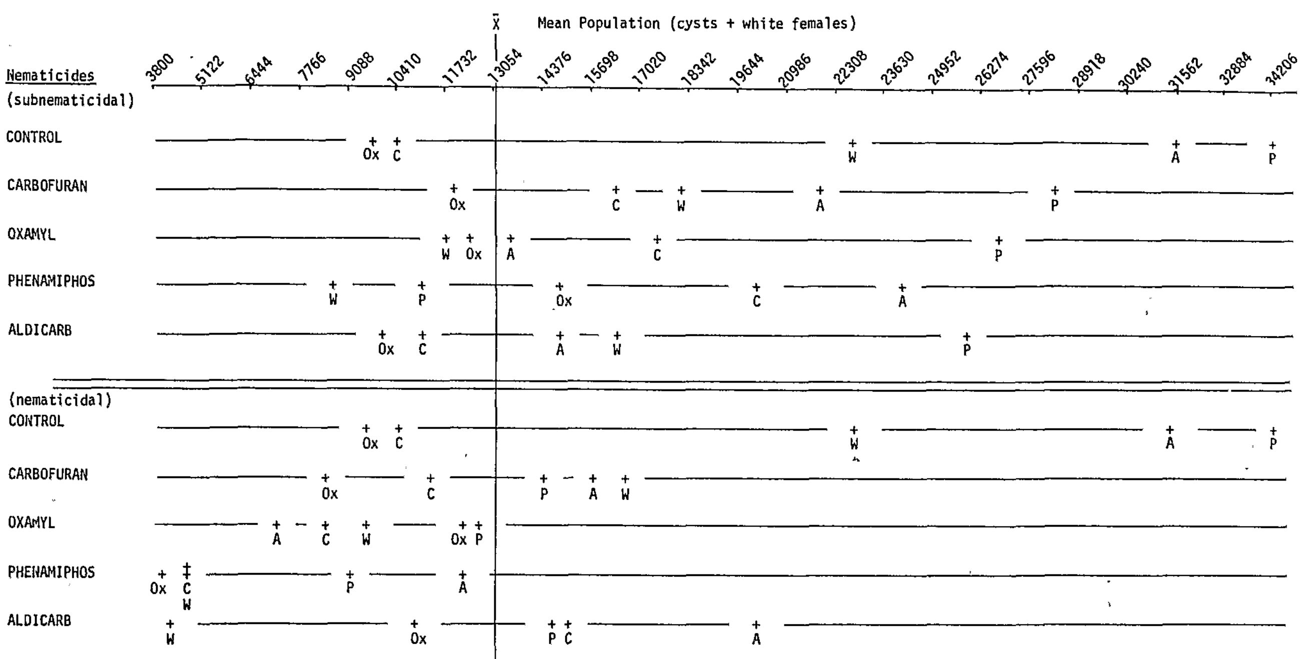
Discussion

Discussion of the complexity of behavioral modification encountered in stressing by nematicidal treatments of *H. schachtii* can be facilitated by diagrammatic representations suggesting trends and providing perspective : Figure 1 illustrates the nature of responses to nematicidal, subnematicidal and control treatments for each stressed population; Figure 2 serves to illustrate the responses of stressed populations to individual NFN treatments. Prolonged stressing of *H. schachtii* with sublethal doses of NFN modified its reproductive capacity (Fig. 2, CTL). Untreated C-S-P and Ox-S-P reproductive capacity was inhibited while A-S-P and P-S-P achieved population levels on the order of three times greater than the other stressed populations. The wild population level was intermediate. These results suggest the existence of at least two mechanisms affecting the reproductive capacity of *Heterodera schachtii.* Moreover, the trends indicate (Fig. 1) that with C-S-P and Ox-S-P, treated with any subnematicidal dose, populations increased with P_s having the largest effect. In contrast, P-S-P and A-S-P with stimulated reproductive capacity, any subnematicidal treatment



Abbreviations are as follows: CTL=Control, C=Carbofuran, Ox=Oxamyl, P=Phenamiphos, A=Aldicarb, subscript s=subnematicidal and subscript n=nematicidal. The wild population has no previous history of being treated with nonfumigant nematicides. Other populations have been stressed monthly with low doses of the respective nonfumigant nematicide.

Fig. 1. Effects on various populations of *Heterodera schachtii* of nonfumigant nematicides.



Abbreviations are as follows: W=Wild Population, C=Carbofuran-Stressed Population, Ox=Oxamyl-Stressed Population, P=Phenamiphos-Stressed Population, A=Aldicarb-Stressed Population. The wild population has no previous history of treatment with nonfumigant nematicides (NFN). Other populations have been stressed monthly with low doses of the respective NFN. The nematicidal NFN concentration is a ten-fold increase over the subnematicidal concentration.

Fig. 2. Responses of various populations of *Heterodera schachtii* to nonfumigant nematicides.

lowered the resultant population with P_s being most effective with P-S-P and Ox_s and A_s being most effective with A-S-P.

With the increase in concentrations to nematicidal levels, some changes were noted. Aldicarb at subnematicidal levels (A_s) generated virtually no effect on population increase, whereas nematicidal doses (A_n) were very effective in controlling the population increase as was P_n. In contrast, Ox_n and C_n were essentially no more effective than the respective subnematicidal concentrations in controlling population increase of W-P (Fig. 1, bottom section). The response to an increased concentration of phenamiphos was striking; P_s was most effective at increasing populations, whereas P_n was the most effective at restricting population increases. All other NFN at nematicidal concentrations clustered around the respective controls; it appeared with these two stressed populations that the dominant factor was the reduced reproductive capacity incurred during stressing. With P-S-P and A-S-P, the increase in concentration of NFN tented to a greater inhibition of population increases with several exceptions, e.g., P-S-P, $P_n = P_s$; $Ox_n > Ox_s$, $C_n > C_s$ and $A_n > A_s$ and for A-S-P $Ox_n > Ox_s$, $P_n > P_s$, $C_n > C_s$, $A_n < A_s$. It appears that stressing an *H. schachtii* population with carbofuran or oxamyl offered no protection, and it remained as susceptible to nematicidal phenamiphos as the wild population, irrespective of other physiological properties incurred by the populations during stressing. In contrast, stressing *H. schachtii* with phenamiphos or aldicarb appeared to sensitize the nematodes to nematicidal concentrations of NFN with respect to their untreated controls. The observation that the populations achieved by phenamiphos and aldicarb stressing in comparison to carbofuran and oxamyl stressing had shifted upwards in nematicidal NFN treatments suggested that the enhanced reproductive capacity developed during stressing remained an important factor. The enhanced reproductive capacity of phenamiphos and aldicarb stressing of *H. schachtii* seen in the controls may be characteristic of phenamiphos stressing, because similar behavior was observed with *Criconemella xenoplax* (Yamashita & Viglierchio, 1987 *b*) and *Xiphinema index* left untreated nearly two years (Yamashita & Viglierchio, 1986 *b*). In the case of *X. index*, phenamiphos stressing resulted in a selection of a population with a long-lived characteristic (at least two years). It remains for future experiments to establish whether the qualities arbitrarily termed " depressed reproductive capacity " for carbofuran and oxamyl-stressed populations and " enhanced reproductive capacity " for phenamiphos and aldicarb-stressed populations persist long-term. The implication of these greenhouse findings is that in the field situation, mismanagement can effect a field population of nematodes with characteristics less amenable to control by chemicals.

The concept of protection incorporating the notions of resistance, cross-resistance and habituation afforded by stressed nematode populations applies to *H. schachtii*, however not as prominently as with other tested species. Habituation, expressed as a requirement for NFN to generate higher population levels, was evident with C-S-P treated with P_s and perhaps C_s, Ox_s and A_n, while with Ox-S-P, perhaps P_s. Other forms of resistance or cross-resistance were minor or questionable, e.g., P-S-P treated with A_s, Ox_s and C_s gave rise to population levels greater than similar treatments of W-P, although less than P-S-P, CTL. The higher population levels of the subnematicidal treatments may have simply been a reflection of the increased reproductive capacity of the stressed population.

Increased susceptibility due to the stressing appeared not to occur with *H. schachtii*. An apparent increase of susceptibility of A-S-P to Ox_n may not have been real; the population levels after Ox_n treatments of all five populations appeared essentially the same. The results are similar to those obtained with P_n treatments of Ox-S-P, C-S-P and W-P which were also the same (Fig. 1, bottom section).

In summary, a wildtype population of *H. schachtii*, like other nematodes investigated, possesses the capacity to alter its behavioral characteristics in response to persistent stress. As a consequence of the modification processes, some of the adaptive characteristics among the different nematode species appeared similar, other did not; the specifics however must await future investigations. For an effective long-term nematode management strategy, optional protocols with different balances of early results and long-term consequences are desirable to achieve a measure of control and avoid adaptation. This notion is equally valid for sublethal applications involving frequent low doses or slow-release granules as well as the less frequent nematicidal applications. There is substantial risk in a management strategy that simply considers only the population levels appearing at the low end of the population scale (Fig. 2), without integrating into the total plan the results of situations at the high end of the population scale as well as the outcome of the intervening sequences.

References

Boehm, N. M. (1986). The development of controlled release granular nematicidal formulations for use in annual and perennial cropping systems. *J. Nematol.*, 18 : 600 [Abstr.].

MacDonald, D. H. (1976). Effects of continued application of aldicarb to greenhouses infested with *Paratylenchus hamatus*. *J. Nematol.*, 8 : 293-294.

National Research Council (1986). *Pesticide resistance : Strategies and tactics for management.* Washington, DC, National Academy Press, 471 p.

Smolik, J. D. (1978). Influence of previous insecticidal use on the ability of carbofuran to control nematode populations in corn and effects on corn yield. *Pl. Dis. Reptr*, 62 : 95-99.

THORNE, G. (1961). *Principles of Nematology*. New York, McGraw Hill, 553 p.

VIGLIERCHIO, D. R. & BROWN, S. M. (1989). *In vitro* testing for nonfumigant resistance in *Heterodera schachtii. Revue Nématol.*, 12 : 139-143.

YAMASHITA, T. T. & VIGLIERCHIO, D. R. (1986*a*). The behavior of nonfumigant nematicide-stressed, unstressed and wild populations of *Pratylenchus vulnus* cultured on grapevines and bean plants. *Revue Nématol.*, 9 : 267-276.

YAMASHITA, T. T. & VIGLIERCHIO, D. R. (1986*b*). Variations in stability of behavioral changes in nonfumigant nematicide-stressed populations of *Xiphinema index* following release from subnematicidal stress. *Revue Nématol.*, 9 : 377-383.

YAMASHITA, T. T. & VIGLIERCHIO, D. R. (1986*c*). *In vitro* testing for nonfumigant nematicide resistance in *Meloidogyne incognita* and *Pratylenchus vulnus. Revue Nématol.*, 9 : 385-390.

YAMASHITA, T. T. & VIGLIERCHIO, D. R. (1987*a*). *In vitro* testing for nonfumigant nematicide resistance in *Xiphinema index. Revue Nématol.*, 10 : 75-79.

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YAMASHITA, T. T. & VIGLIERCHIO, D. R. (1987*b*). Induction of short-term tolerance to nonfumigant nematicides in wild populations of *Xiphinema index* and *Pratylenchus vulnus. Revue Nématol.*, 10 : 93-100.

YAMASHITA, T. T. & VIGLIERCHIO, D. R. (1987*c*). Field resistance to nonfumigant nematicides in *Xiphinema index* and *Meloidogyne incognita. Revue Nématol.*, 10 : 327-32.

YAMASHITA, T. T. & VIGLIERCHIO, D. R. (1987*d*). Induction of short-term tolerance to nonfumigant nematicides in stressed and unstressed populations of *Xiphinema index. Revue Nématol.*, 10 : 233-240.

YAMASHITA, T. T., VIGLIERCHIO, D. R. & KUO, F. F. (1988). Nonfumigant nematicide conditioned populations of *Criconemella xenoplax* and their responses to subsequent treatments. *Revue Nématol.*, 11 : 429-435.

YAMASHITA, T. T., VIGLIERCHIO, D. R. & SCHMITT, R. V. (1986). Responses of nematodes to nematicidal applications following extended exposures to subnematicidal stress. *Revue Nématol.*, 9 : 49-60.