# The role of microbial populations from long-terme nonfumigant nematicide-treated soils on *Heterodera schachtii* nematicide trials

Tom T. YAMASHITA*, David R. VIGLIERCHIO and Frances FAN-KUO

*Division of Nematology, University of California, Davis, CA 95616, USA*

SUMMARY

Stock cultures of *Heterodera schachtii* were treated monthly with low doses of nonfumigant nematicide (NFN) for three continuous years (stressed populations). Soils from these stressed cultures and from a wild population culture were washed and the aqueous supernatant was freed of nematodes and eggs (leachings). Following incubation of one-half of the leachings with 10 % AC Medium for 24 hours, the microbial populations were increased approximately three-thousand-fold. The other half of the leachings were kept at 5° for 24 hours. These leachings were then added to test posts inoculated with wild-type *H. schachtii* (J2) juveniles. Two days later the pots were treated with NFN once daily for three consecutive days. Extraction of nematodes two months later indicated no detectable qualitative differences between the microbial populations in NFN-stressed *vs* wild-stock nematode cultures. However, leachings from NFN-stressed cultures appeared to have influential factors of unknown origin. In the absence of NFN treatment, certain leaching components stimulated nematode activity. Conversely, when treated, certain leaching components appeared to have both an additive and/or synergistic reaction with the applied NFN.

RÉSUMÉ

*Rôle joué dans les essais nématicides contre* Heterodera schachtii *par les populations microbiennes des sols traités pendant de longues périodes avec un nématicide non fumigant*

Des élevages de base d'*Heterodera schachtii* ont été traités tous les mois avec de faibles doses d'un nématicide non fumigant (NNF) pendant une période de trois ans (populations sensibilisées). Le sol correspondant à ces populations sensibilisées, et celui provenant d'élevages sauvages sont mis en suspension dans de l'eau; la phase liquide supérieure (ou « filtrat ») est ensuite débarrassée des nématodes et de leurs œufs. Après incubation pendant 24 heures de la moitié du filtrat, additionnée de 10 % du milieu AC, le peuplement microbien croît d'un facteur approximatif de 3 000. L'autre moitié du filtrat est laissée 24 heures à 5°. Les deux types de filtrat sont ajoutés à des pots inoculés avec des juvéniles (J2) de la souche sauvage d'*H. schachtii*. Deux jours après les pots sont traités avec un NNF une fois par jour pendant trois jours. L'extraction des nématodes après deux mois ne permet de détecter aucune différence qualitative entre les peuplements microbiens liés aux élevages des populations sensibilisées ou sauvages. Cependant les filtrats provenant des élevages sensibilisés paraissent recéler des facteurs actifs d'origine inconnue. En l'absence de traitement avec un NNF, certains composants du filtrat stimulent l'activité du nématode. Réciproquement, s'il y a traitement, certains composants du filtrat paraissent avoir une action complémentaire et/ou synergétique vis-à-vis du NNF.

An increasing concern for environmental safety has prompted extensive studies into the role played by microorganisms in pesticide decomposition (Helling, Kearney & Alexander, 1971; Harris, 1972; Laveglia & Dahm, 1977; Woodcock, 1978). This concern has grown with the increased emphasis upon soil-applied nonfumigant organophosphates and carbamates. There is a general agreement on chemical decomposition and the role of several edaphic factors on nonfumigant nema-

others have found no correlation between carbofuran degradation and microbial enrichment of the soil (Venkateswarlu, Siddarama Gowda & Sethunathan, 1977; Ahmad, Walgenback & Sutter, 1979). Furthermore, the various NFN's appear to have different effects on the soil microorganism population (Mathur, Hamilton & Vrain, 1980). To complicate the picture, microbial populations previously observed to degrade one particular NFN, may adapt to degrade related and unrelated than three years. Casual observations, via dilution plating, had also indicated differential microbial population levels in the various NFN-stressed nematode stock cultures.

The significance of microbial-mediated NFN degradation extends to several practical considerations, one of which involves NFN persistence and, thus, the existence of selective pressures for development of NFN resistance. If long-term monthly stressing of nematode stock cultures could also condition the microbial population for an enhanced decomposition of the NFN, the application of a soil-free suspension (leaching) from these stock cultures to nematode-inoculated test pots could possibly provide a protection from subsequent NFN treatments. This phenomenon was observed in a previous study (Yamashita, Viglierchio & Schmitt, 1986). Of equal interest would be the differential effects of leachings from the various stressed nematode stock cultures. If the application of a particular NFN can alter the of leachings from various NFN-stressed cultures of *H. schachtii* in comparison to leachings taken from a nematode stock population with no previous history of NFN stressing.

## Materials and methods

Seedlings of sugarbeets *(Beta vulgaris)* were started in 12.5 cm sterilized clay pots with an autoclaved mixture of two parts river sand to one part fine white sand. Following two months' growth, all test pots were inoculated with an aliquant of 2 000 freshly-extracted J2 stages of *H. schachtii* having no previous history of NFN treatments (wild population). One week following inoculation 200 test pots were divided into five groups. Each group (40 pots) was, then, drenched with 150 ml of leachings taken from *H. schachtii* stock cultures of either a : a) wild population (W-P); b) carbofuran-stressed population (C-S-P); c) oxamyl-stressed population (Ox-S-P), d) phenamiphos-stressed population (P-S-P); e) aldicarb-stressed populations (A-S-P). Stressed populations were generated by treating an initial wildtype population (no previous history of NFN treatment) with

(20 pots) was drenched with amended leachings; the remaining 20 test received unamended leachings.

Two days after the addition of leachings, test pots were treated for three successive days with either water (control) or subnematicidal levels ($NFN_s$) for unamended leachings and a ten-fold higher concentration ($NFN_n$) for amended leachings. The following NFN concentrations were used for treatments : carbofuran and oxamyl ($NFN_s$ = 0.008 mM, $NFN_n$ = 0.08 mM); phenamiphos and aldicarb ($NFN_s$ = 0.0012 µM, $NFN_n$ = 0.012 mM). The methods of treatment were as outlined in an earlier study (Yamashita, Viglierchio & Schmitt, 1986).

An additional group of pots, which had been drenched with amended leachings, were treated with nematicidal concentrations of NFN's made up in 50 mM phosphate buffer, adjusted to pH 6.0. During incubation of leachings with AC Medium, microbial populations generated ammoniacal odors. The use of the acidic phosphate buffer was an attempt to prevent or minimize spontaneous chemical breakdown of NFN's, which might have been mediated by an increase in pH.

Two months following treatment, all pots were harvested. Sand and roots were washed three times successively and the suspension passed through an 833 µm and onto a 246 µm pore sieve. In addition, roots were gently cleaned under a gentle stream of water (over the 246 µm sieve) to catch dislodged cysts and white females. All cysts and white females, caught on the 246 µm sieve, were later dried on filter paper then counted. Fibrous, nonstorage roots were blotted dry and the weights recorded. Populations were evaluated following a $Log_{10}$ (cysts + white females/gr root) transformation, where each treatment had four replicates. ANOVA was conducted using a three-factor univariate analysis. Duncan's Multiple Range Test was used for mean comparisons with an upper significance level of 5 %.

## Results

There were no appreciable differences observed at a

Table 1

Isolated effects of different factors on *Heterodera schachtii*

| | | | |
|---|---|---|---|
| A. Effects of Leachings | | B. Effects of nematicides | |
| WILD | 200 a | CONTROL | 301 b |
| CARBOFURAN | 259 a | CARBOFURAN | 196 ab |
| OXAMYL | 225 a | OXAMYL | 160 a |
| PHENAMIPHOS | 163 a | PHENAMIPHOS | 255 ab |
| ALDICARB | 213 a | ALDICARB | 169 a |
| C. Effects of Incubating Leachings in AC Medium | | D. Effects of Subnematicidal *vs* Nematicidal Levels of NFN | |
| w/o AC Medium | 330 a | Subnematicidal | 358 b |
| w/AC Medium | 133 a | Nematicidal | 110 a |
| E. Effects of Acidified Buffer | | | |
| w/o Buffer | 123 a | | |
| w/Buffer | 142 a | | |

Numbers represent the mean populations of (cysts + white females/gram of root) for the respective variables. Those not followed by a common letter are different at a significance level of 5 % or less.

Leachings, AC Medium and acidified buffer did not appreciably affect the population levels (Tab. 1, A, C, E). For example, all test pots which were drenched in amended or unamended leachings or left untreated (controls) were statistically similar (Tab. 2). Furthermore, when unamended and amended controls were compared (e.g., WILD w/o AC Medium *vs* WILD w/AC Medium) it was apparent that the incubation of leachings with AC Medium and concomitant increases in microbial populations had no marked effect on nematode population levels (Tab. 2).

### Leachings without AC Medium

Trends which may have practical importance are depicted in Figures 1 and 2. Untreated (control) pots, receiving leachings from carbofuran, oxamyl and phenamiphos stock cultures tended towards generally high population levels, while untreated pots drenched with wild and aldicarb stock culture leachings produced the lowest nematode numbers (Figs 1, 2). Of every leaching without AC, except for oxamyl, subnematicidal carbofuran ($C_s$) treatments yielded either the highest or second to highest populations (Fig. 1). It appeared as though some form of synergistic activity between the oxamyl leachings and $C_s$ treatment had occurred. Pots drenched with leachings from oxamyl stock cultures produced the largest number of nematodes except when treated with carbofuran, which gave the lowest number. In a similar manner, pots that had received leachings from C-S-P stock cultures gave consistently high nematode numbers except when treated with aldicarb. Conversely, pots which had been drenched with leachings from A-S-P stock cultures tended to have low numbers (Fig. 2, subnematicidal series A).

### Leachings with AC Medium

Untreated pots previously drenched with leachings from C-S-P and A-S-P cultures yielded the highest nematode numbers (Fig. 2 w/AC Medium, B). While the leachings from C-S-P were consistent with the results without AC, the leachings from A-S-P were a complete reversal from results without AC Medium. Furthermore, incubating leachings from Ox-S-P and P-S-P stock cultures allowed for the development of some factor(s) that reduced nematode population levels (Fig. 2 B), in contrast to the effects obtained in pots without AC Medium (Fig. 2 A).

Pots receiving amended leachings from the W-P stock cultures appeared unaffected by all $NFN_n$ treatments (Tab. 2, WILD w/AC Medium). However, pots drenched with amended leachings from all stressed population stock cultures were significantly reduced in nematode numbers by one or more $NFN_n$ treatments (Tab. 2, w/AC Medium series) : a) carbofuran w/AC; CTL = 446 *vs* $C_n$ = 104, $C_n$ + Buffer = 90 and $Ox_n$ = 98, b) oxamyl w/AC; CTL = 215 *vs* $C_n$ = 33, c) phenamiphos w/AC; CTL = 230 *vs* $A_n$ = 36 and $Ox_n$ = 56 (7 % level), d) aldicarb w/AC; CTL = 516 *vs* $Ox_n$ = 83.

## Discussion

Preliminary casual observations (dilution plating), indicating a several-fold greater microbial population in stressed than in wild population stock cultures, were consistent with results of field applications of NFN's on the microbial populations (Mathur, Hamilton & Vrain, 1980). The amendment of a 10 % AC Broth (designed for culturing aerobic microorganisms) to leachings, though in part arbitrary, was intended to provide a moderate enhancement of the aerobic segment of the microbial population. Following the 24 hour incubation of leachings with AC Medium, dilution plating indicated an approximate three-thousand-fold increase in the microbial population, largely represented by various species of bacteria and very few fungi.

There was an absence of significant differences either within or between control pots drenched in leachings with or without AC Medium. Microbial numbers and effects of leachings, AC Medium and buffer exhibited a negligible impact on nematode populations; however, nematicidal concentrations proved significant. The interactions between leaching, AC Medium and nematicide reveal possible trends towards certain unique qualities in the various leachings.

Table 2

Microbial population effects on control of *Heterodera schachtii* with nonfumigant nematicides

| Leachings | *Nematicide Treatment* | | | | |
|---|---|---|---|---|---|
| | *Control* | *Carbofuran* | *Oxamyl* | *Phenamiphos* | *Aldicarb* |
| WILD w/o AC Medium | 170 cdefghijk | 442 hijk | 299 defghijk | 303 defghijk | 233 cdefghijk |
| CARBOFURAN w/o AC Medium | 493 ijk | 514 jk | 415 ghijk | 603 k | 212 cdefghijk |
| OXAMYL w/o AC Medium | 532 jk | 299 defghijk | 545 jk | 646 k | 401 ghijk |
| PHENAMIPHOS w/o AC Medium | 461 ijk | 389 fghijk | 191 cdefghijk | 229 cdefghijk | 368 efghijk |
| ALDICARB w/o AC Medium | 156 cdefghijk | 330 defghijk | 253 defghijk | 182 cdefghijk | 289 defghijk |
| WILD w/AC Medium | 167 cdefghijk *180 cdefghijk | 173 cdefghijk | 93 abcdef | 298 defghijk | 88 abcde |
| CARBOFURAN w/AC Medium | 446 hijk | 104 abcdefgh *90 abcde | 98 abcdefg | 165 cdefghijk | 132 abcdefghij |
| OXAMYL w/AC Medium | 215 cdefghijk | 33 a | 80 abcd *169 cdefghijk | 201 cdefghijk | 128 abcdefghij |
| PHENAMIPHOS w/AC Medium | 230 cdefghijk | 88 abcde | 55 abc | 117 abcdefghi *134 bcdefghij | 36 ab |
| ALDICARB w/AC Medium | 516 ijk | 186 cdefghijk | 83 abcd | 205 cdefghijk | 176 cdefghijk |

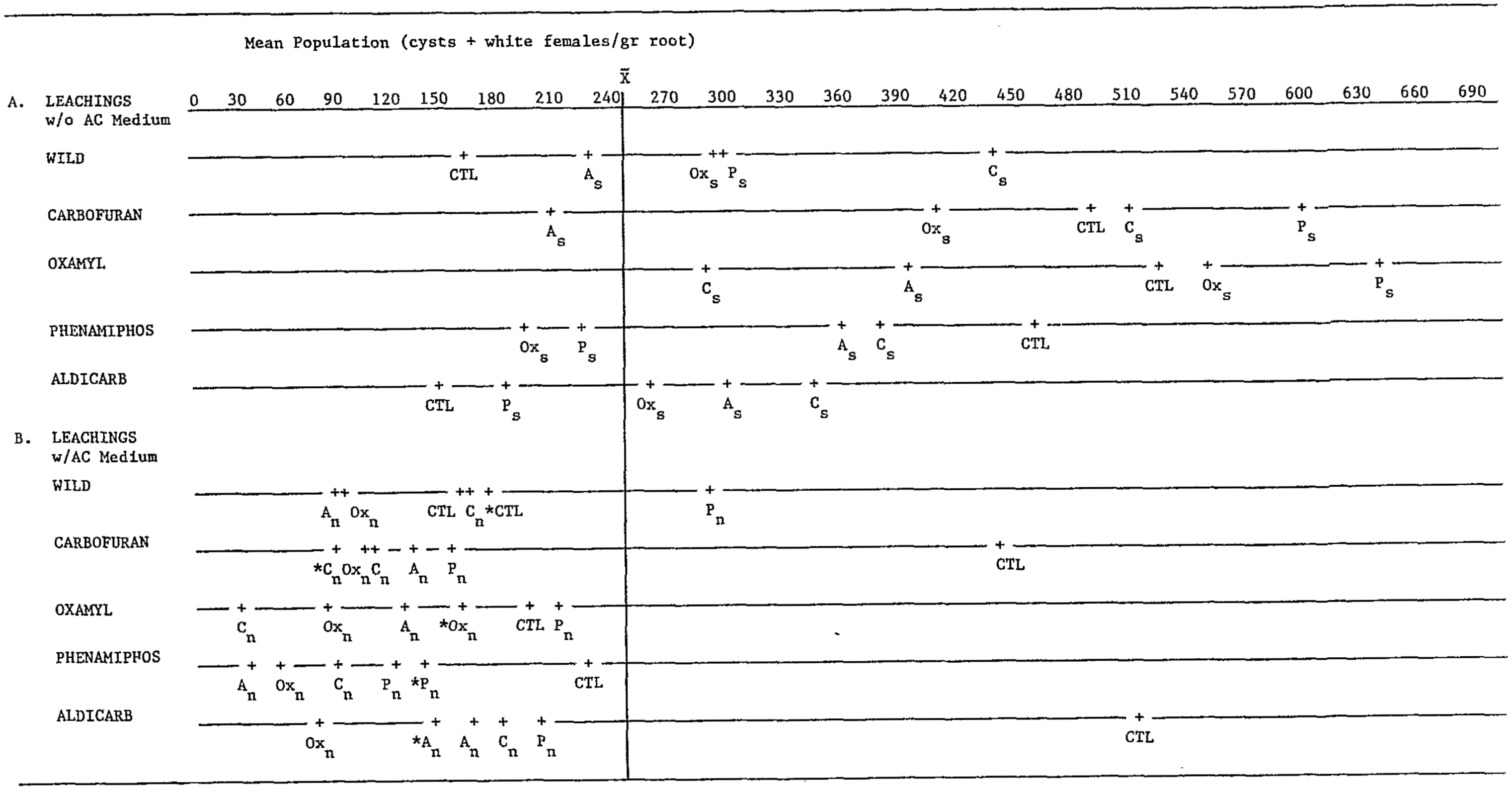


Fig. 1. *Heterodera schachtii* population levels of leachings as modified by treatments.

Leachings in (B) were incubated for 24 hours following the addition of 3.2 gr/L AC Broth Medium (Difco Laboratories, Detroit, Mighigan) prior to drenching the test pots. Test pots receiving leachings without AC Medium were treated with subnematicidal elvels, while those receiving leachings with AC Medium were treated with a ten-fold higher concentration of nonfumigant nematicide. Treatments are abbreviated as follows : CTL = Control, C = Carbofuran, Ox = Oxamyl, P = Phenamiphos, A = Aldicarb, subscript s = subnematicidal, subscript n = nematicidal or 10 × concentration of s. (*) = nonfumigant nematicide applied in 509 mM phosphate buffer, pH = 6.0.

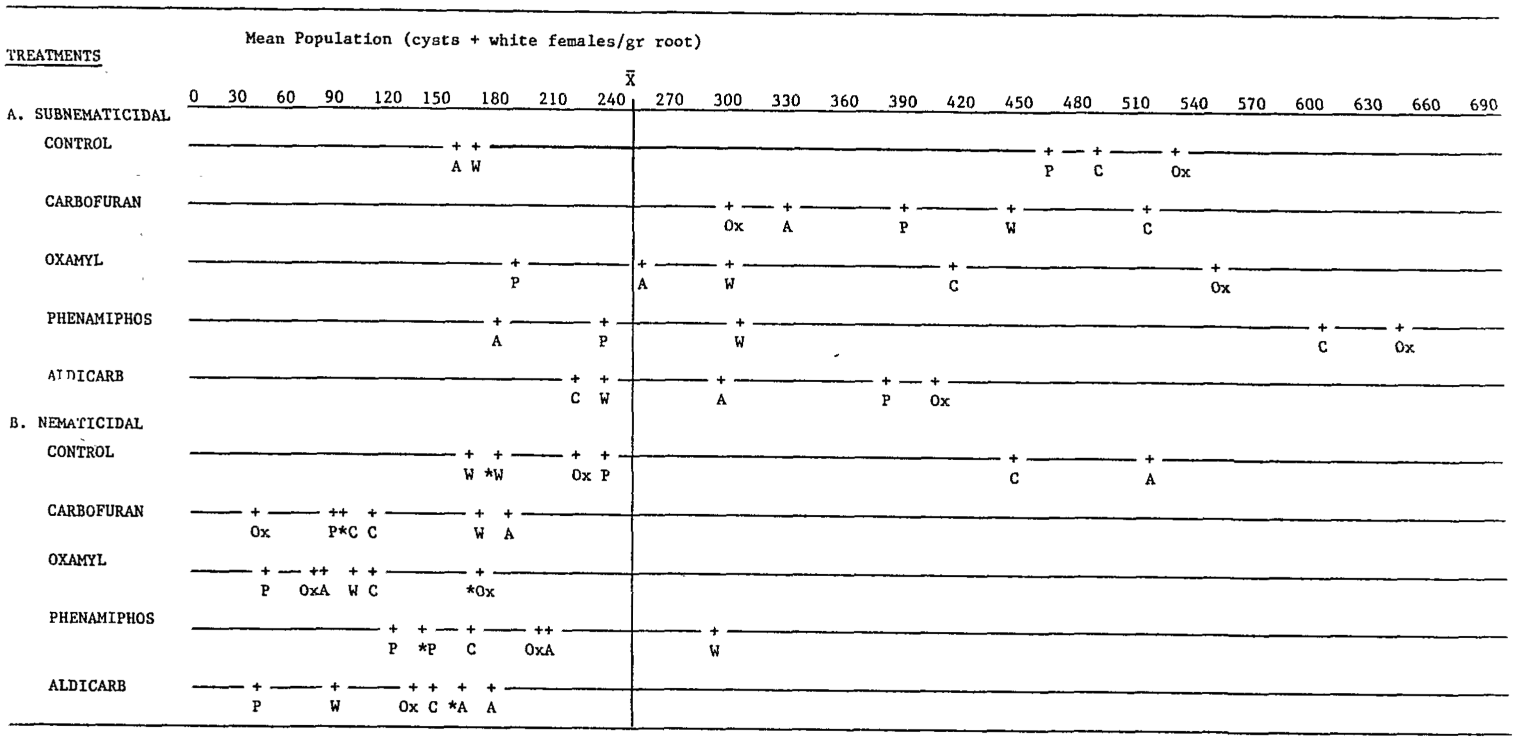


Fig. 2. *Heterodera schachtii* population levels of treatments as modified by leachings.

Leachings in (B) were incubated for 24 hours following the addition of 3.2 gr/L AC Broth Medium (Difco Laboratories, Detroit, Michigan) prior to drenching the test pots. Test pots receiving leachings without AC Medium were treated with subnematicidal levels, while those receiving leachings with AC Medium were treated with a ten-fold higher concentration of nonfumigant nematicide. Leachings are abbreviated as follows : W = Wild Populations, C = Carbofuran-Stressed Population, Ox = Oxamyl-Stressed Population, P = Phenamiphos-Stressed Population, A = Aldicarb-Stressed Population. (*) = nonfumigant nematicide applied in 50 mM phosphate buffer, pH = 6.0.

leachings taken from NFN-stressed population stock cultures (Yamashita, Viglierchio & Schmitt, 1986), Ox-S-P leachings on *X. index* and Ox-S-P, C-S-P and P-S-P leachings applied to *M. incognita* pots resulted in markedly lower nematode numbers than undrenched pots following NFN treatments. However, *X. index* pots drenched in C-S-P and P-S-P leachings had higher population levels over undrenched pots following NFN treatment. The present study is consistent with a previous one (Yamashita & Viglierchio, 1986) for a stimulatory response in nematodes to subnematicidal levels of NFN. Perhaps this may be suspected in the apparent stimulatory response following drenches with C-S-P, Ox-S-P and P-S-P leachings without AC Medium (controls), i.e., low levels of NFN residues in leachings may have been complicating the response. Steele (1977) observed a stimulatory action of low aldicarb and carbofuran concentrations (*ca.* 1 µg/ml) on

insignificant, d) certain factors in stock culture leachings appeared to react synergistically with nematicidal levels of certain NFN's for enhanced control of nematodes, e) certain factors in stock culture leachings appeared to induce stimulatory-like responses in nematodes, f) incubation of certain leachings for 24 hours in 10 % AC Medium appeared to reveal components which were manifested by an increase or decrease in corresponding control populations, g) at concentrations used, oxamyl and aldicarb, with relatively higher systemic activity, appeared to provide better control than did carbofuran or phenamiphos.

The observations of NFN resistance, indifference, habituation and increased susceptibility in greenhouse (Yamashita, Viglierchio & Schmitt, 1986), laboratory (Yamashita & Viglierchio, 1987*a*) and field studies (Yamashita & Viglierchio, 1987*b*) in various nematode species have prompted a major concern for the contribution

GETZIN, L. W. (1973). Persistence and degradation of carbofuran in soil. *Environm. Ent.*, 2 : 461-467.

GIEBEL, J. (1979). [Biochemical mechanisms of plant resistance to nematodes.] *Prace Nauk, Instyt. Ochony Roslin, Poznan*, 21 : 189-212.

GORDER, G. W., DAHM, P. A. & TOLLEFSON, J. J. (1982). Carbofuran persistence in cornfield soils. *J. econ. Ent.*, 75 : 637-642.

HARRIS, C. R. (1972). Factors influencing the effectiveness of soil insecticides. *A. Rev. Ent.*, 17 : 177-198.

HELLING, C. S., KEARNEY, P. C. & ALEXANDER, M. (1971). Behavior of pesticides in soils. In : Brady, N. C. (Ed.). *Advances in Agronomy. Vol. 23*, New York, Academic Press : 147-240.

JOHNSON, A. W., ROHDE, W. A., DOWLER, C. C., GLAZE, N. C. & WRIGHT, W. C. (1981). Influence of water and soil temperature on the concentration and efficacy of phenamiphos or control of root-knot nematodes. *J. Nematol.*, 13 : 148-153.

KAUFMAN, D. D. & EDWARDS, D. F. (1982). Pesticide/microbe interaction effects on persistence of pesticides in soil. Comm. *Vth Intern. Congr. Pesticide Chemistry (IUPAC), Aug. 29-Sept. 4, 1982, Kyoto, Japan.*

LEVEGLIA, J. & DAHM, P. A. (1977). Degradation of organophosphorous and carbamate insecticides in the soil and by soil microorganisms. *A. Rev. Ent.*, 22 : 403-503.

MATHUR, S. P., HAMILTON, H. A. & VRAIN, T. C. (1980). Influence of some field-applied nematicides on microflora and mineral nutrients in an organic soil. *J. environm. Sci. & Health*, 15 : 61-76.

MILNE, D. L., VANLELYVELD, L. J., VILLIERS, E. A. (1977). The response of peroxidase and indoleacetic acid oxidase in pineapple roots to foliar application of phenamiphos. *Agrochemophysica*, 9 : 65-69.

OZAKI, K. (1983). Suppression of resistance through synergistic combinations with emphasis on planthoppers and leafhoppers infesting rice in Japan. In : Georghiou, G. P. & Saito, T. (Eds). *Pest Resistance to Pesticides*, New York, Plenum Press : 595-613.

SARAH, J. L. (1981). Utilisation de nématicides endothérapiques dans la lutte contre *Pratylenchus brachyurus* (Godfrey) (Nematoda, Pratylenchidae) en culture d'ananas. III-Effets secondaires d'applications foliaires sur la réponse au traitement d'induction florale et sur la floraison. *Fruits* 35 : 491-500.

SMELT, A. E. (1983). Effects of selected nematicides on hatching of *Heterodera schachtii*. *J. Nematol.*, 15 : 467-473.

SMELT, J. H., LEISTRA, M., HOUX, N. W. H. & DEKKER, A. (1978*a*). Conversion rates of aldicarb and its oxidation products in soils. II. Aldicarb sulphoxide. *Pesticide Sci.*, 9 : 286-292.

SMELT, J. H., LEISTRA, M., HOUX, N. W. H. & DEKKER, A. (1978*b*). Conversion rates of aldicarb and its oxidation products in soils. III. Aldicarb. *Pesticide Sci.*, 9 : 293-300.

STEELE, A. E. (1977). Effects of selected carbamate and organophosphate nematicides on hatching and emergence of *Heterodera schachtii*. *J. Nematol.*, 9 : 149-154.

STEIFERT, J. & DAVIDEK, J. (1978). The carbamoylation and spontaneous reactivation of the cabbage carboxylesterase after interaction with carbofuran. *Pesticide Biochem. Physiol.*, 8 : 10-14.

VENKATESWARLU, K., SIDDARAMA GOWDA, T. K. & SETHUNATHAN, N. (1977). Persistence and biodegradation of carbofuran in flooded soils. *J. agric. & Food Chem.*, 25 : 533-536.

VIGLIERCHIO, D. R. BROWN, S. M., & FAN-KUO, F. F. (1989). Adaptive responses of *Heterodera schachtii* populations to nematicidal applications of nonfumigant nematicides after stressing with sublethal doses. *Revue Nématol.* 12 (in press).

WILLIAMS, I. H., PEPIN, H. S. & BROWN, M. J. (1976). Degradation of carbofuran by soil microorganisms. *Bull. environm. Toxicol.*, 15 : 244-249.

WOODCOCK, D. (1978). Microbial degradation of fungicides, fumigants and nematicides. In Hall, I. R. & WRIGHT, S. J. L. (Eds). *Pesticides Microbiology, Microbial Aspects of Pesticide Behavior in the Environment.* New York, Academic Press : 731-780.

YAMAMOTO, I., TAKAHASHI, Y. & KYOMURA, N. (1983). Suppression of altered acetylcholinesterase of the green rice leafhopper by N-propyl and N-methyl carbamate combinations. In Georghiou, G. P. & SAITO, T. (Eds), *Pest Resistance to Pesticides.* New York; Plenum Press : 479-594.

YAMASHITA, T. T. & VIGLIERCHIO, D. R. (1986). Variations in the 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. (1987*a*). In vitro testing for nonfumigant nematicide resistance in *Xiphinema index*. *Revue Nématol.*, 10 : 75-79.

YAMASHITA, T. T. & VIGLIERCHIO, D. R. (1987*b*). Field resistance to nonfumigant nematicides in *Xiphinema index* and *Meloidogyne incognita*. *Revue Nématol.* 10 : 327-332.

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

*Accepté pour publication le 22 septembre 1987.*