Tribune

THE IMPACT OF NEMATODE ADAPTABILITY ON THE PROSPECT FOR THEIR CONTROL

David R. VIGLIERCHIO

Department of Nematology, University of California, Davis, CA 95616, USA.

Nature evolved a way to cope with change early in its history, perhaps even before the appearance of life forms. For drastic environmental changes, coping was equally drastic, e.g., the collapse of dinosaur dominance eventually to be replaced by that of mammals. Evolution continues to be fueled by flexibility and adaptability of species; inflexible species unable to adapt to environmental pressures of whatever origin, disappear, an observation consistent with the fossil records. This capacity to adapt is inherent in most organisms; moreover, the environmental stress driving the process is not limited to climatic or habitat modifications, but is general, involving many elements including such factors as pests, diseases, nutrients, hosts, and chemotherapeutics, among others.

Pest-like organisms have been part of the planets' environment for eons. Those that were unable to adapt as had other evolving plant and animal life have disappeared. Those that were flexible evolved into the current complement of weeds, microorganisms, insects, nematodes, and others. Initially, the relationship was simply that of an organism seeking a suitable nutritional source. It is only with the advent of modern man that some of these organismal relationships took on an anthropological hue, i.e., organisms seeking food became pests and live nutritional sources became hosts. Pests that became dominant and overwhelmed the host destroyed the food source and disappeared. Hosts that dominated and overwhelmed the pest caused the pest to disappear. The relationship that has survived is an evolutionary dynamic competitive one in which each component alternately may achieve modest advantage but never becomes overwhelmingly dominant. This adaptive process has probably been active since the paleozoic era. The essential point is that nonadaptability in an organism is tantamount to extinction, consequently the greater the capacity for flexibility and adaptability to any stress, the greater the probability of survival.

It is not surprising, therefore, that the great German chemist, Paul Ehrlich, the father of chemotherapy who envisioned " magic bullets " of drugs to cure many diseases of man, became frustrated with his subsequent observations, that drug resistance had followed the development of new chemotherapeutic agents " like a faithful shadow " (Katner & Ling, 1989). The widespread use of antibiotics during the last half century has dramatically raised the specter of drug resistance development to near catastrophic proportions in modern medicine. In a parallel situation the use of modern pesticides for weed, insect, and plant pathogen control resulted in dramatic increases in pest resistance to the point that hundreds of pesticides have become useless (Anon., 1986). Until recently, nematodes had been essentially ignored with respect to a general capacity to adapt to pesticides or other forms of environmental stress. Now there is abundant evidence that nematode populations adapt to nematicides and other forms of environmental stress as do most other organisms. Early warnings with respect to the potential of nematodes to develop resistance to drugs and pesticides beginning some three decades ago with plant-parasitic nematodes (Trujillo-Alvarado, 1956), and followed by animal parasitic nematodes (Drudge *et al.*, 1964), free-living bacterial feeders (Brenner, 1974), greenhouse experiments with plant parasites (MacDonald, 1976), and field trials (Smolik, 1978), were generally ignored. Recent experiments with plant-parasitic nematodes have indicated that behavioral modification of nematodes subject to long-term stress with non-fumigant nematicides (NFN) is a complex phenomenon, rather than a simple development of pesticide resistance. Relations which appear to be involved include among others : 1) Selective pressures from subnematicidal exposures can result in populations with a lower fitness for reproduction; 2) Nematodes can be selected for and/or conditioned to be more sensitive to nematicidal doses; 3) Nematode stressing can yield resistant populations with indifference to nematicidal doses; 4) Nematode stressing can yield resistant populations that show higher reproductive fitness; 5) Nematode stressing yields cross-susceptible and cross resistant populations; 6) Nematode stressing can yield populations that show an habituation to nematicides; 7) There are real differences between nematode species and their response to nematicides. Several of these points are illustrated in a comparative summary (Fig. 1) of stressed populations of five different nematode species treated with nematicidal doses of nonfumigant nematicides. For a complete understanding one needs to refer to the detailed reports (Yamashita,

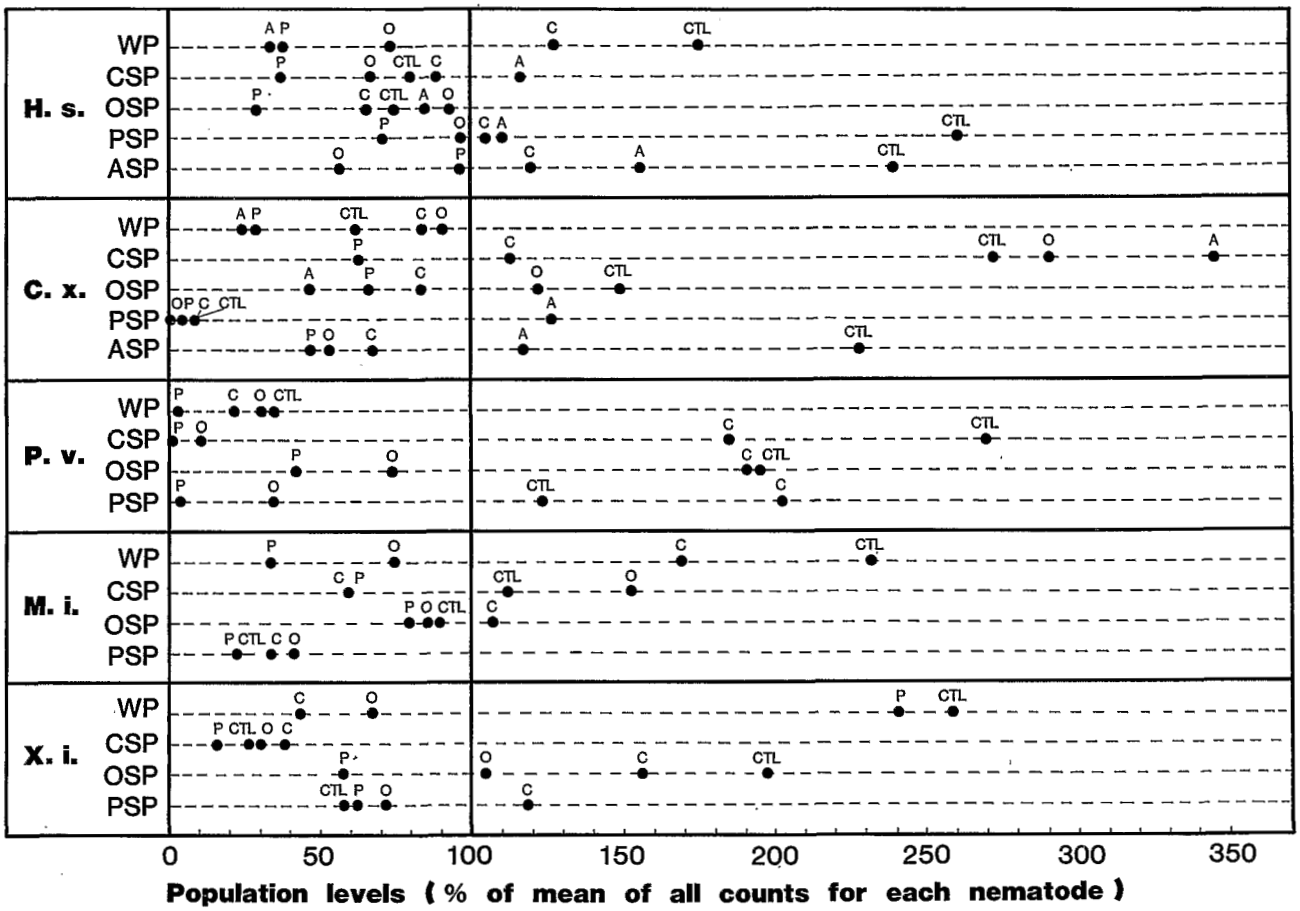

Fig. 1. Population levels as percent of overall mean for each nematode in treatments with different nematicides of nematode populations stressed with different nematicides at sublethal levels. A = Aldicarb, O = Oxamyl, C = Carbofuran, P = Fenamiphos, CTL = control = no treatment, WP = wild untreated population, CSP = carbofuran stressed population, OSP = Oxamyl stressed population, PSP = fenamiphos stressed population, and ASP = Aldicarb stressed population, H.s. = *Heterodera schachtii*, C.x. = *Criconemella xenoplax*, P.v. = *Pratylenchus vulnus*, M.i. = *Meloidogyne incognita*, X.i. = *Xiphinema index*.

Viglierchio & Schmitt, 1986; Yamashita, Viglierchio & Kuo, 1988*b*; Viglierchio, Brown & Kuo, 1989). Nematode species clearly respond differently to the same experimental protocol. While many stressed populations of different species removed from stress (CTL) explode to high population levels, an equal number remains below levels of other treatments. It is also evident that certain stressed populations develop higher population levels with certain nematicidal treatments than others.

Having accepted that nematodes adapt to prolonged pesticide stress, one may ask how long might this adaptation persist before reverting to the normal state with the stress pressures removed? The summary (Table 1) illustrates a trial conducted with *X. index* populations stressed five years and populations stressed three years, then unstressed, i.e., removed from stress for two years. It is clear with *X. index* that the adaptation effected by pesticide stress persists in excess of two years. The most obvious manifestation in this case takes the form of enhanced reproductive capacity (Yamashita & Viglierchio, 1986*b*).

Moreover it can also be asked whether nematodes manifest intermediate or short-term responses to nematicides. One aspect of this question can be evaluated by immersing nematode populations of varied history in high concentrations (in the 0.5 mM range) of NFN, and assessing the response by changes in motility after 24 hours (Fig. 2). The responses of nematodes are widely varied depending not only on species, but also the preconditioning history of the population and the nematicide used for immersion (Yamashita & Viglierchio, 1986*c*, 1987*a*, 1988; Viglierchio & Brown, 1989). The results are in some cases similar to those seen in greenhouse trials, but in other cases not thereby indicating

Table 1

Population levels of different *X. index* populations with different stressing histories, subjected to subnematicidal or no treatments, in terms of percent of populations increase of wild type.

X. index *Population*	*Control*	*Subnematicidal Treatment*		
		C	*O*	*P*
C-S-P	11	11		
O-S-P	76		74	
P-S-P	23			52
C-U-P	67	78		
O-U-P	118		59	
P-U-P	266			135
Wild	100	69	92	97

C = Carbofuran, O = Oxamyl, P = Fenamiphos, C-S-P = C stressed population, O-S-P = O stressed population, P-S-P = P stressed population, C-U-P = C unstressed population, O-U-P = O unstressed population, P-U-P = P unstressed population, wild = never stressed population.

that additional factors are involved in the nematodes' immediate ability to cope with different nematicidal solutions. Since the *in vitro* bioassays involved a somatic musculature reaction, it is evident that these nonfumigant nematicides are not particularly effective for the inhibition of muscle contraction in most nematodes. It is equally evident that in some cases preconditioning provides substantial protection to the activity of a nematicide, but in other cases, an increased sensitivity.

An alternate approach would involve the use of nematode populations of varied history, subjected to an induction treatment with subnematicidal doses of NFN

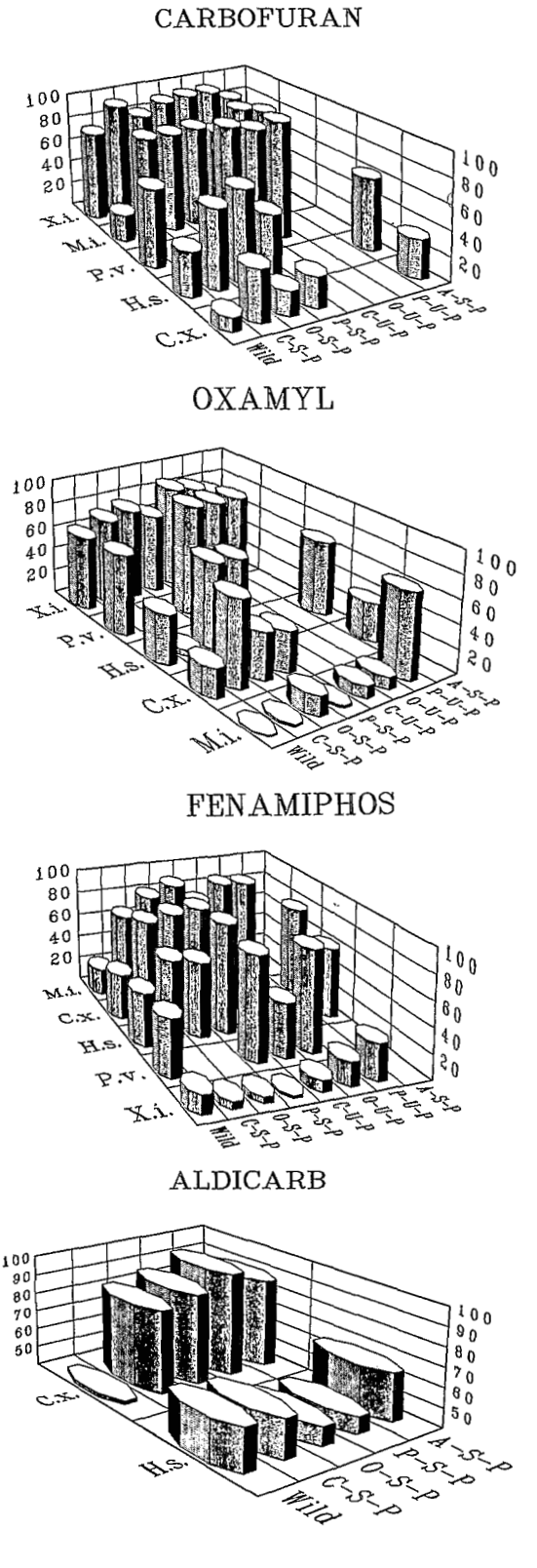

Fig. 2. The percent active of different nematode species from populations variously preconditioned, after *in vitro* immersion at the highest concentration of nematicide tested. P.v. = *Pratylenchus vulnus;* M.i. = *Meloidogyne incognita;* X.i. = *Xiphinema index;* C.x. = *Criconemella xenoplax;* H.s. = *Heterodera schachtii;* wild = never treated population; CSP = carbofuran stressed population; CUP = carbofuran stressed population removed from stress for two years; OSP = Oxamyl stressed population; OUP = Oxamyl stressed population removed from stress for two years; PSP = fenamiphos stressed population; PUP = fenamiphos stressed population removed from stress for two years; ASP = Aldicarb stressed population; *In vitro* carbofuran concentration P.v. = M.i. = X.i. = 0.6 mM, C.x. = H.s. = 1.0 mM; Oxamyl concentrations, P.v. = M.i. = 0.4 mM, C.x. = H.s. = 0.5 mM, X.i. = 0.6 mM; fenamiphos concentrations P.v. = M.i. = C.x. = H.s. = 0.4 mM, X.i. = 0.16 mM; aldicarb concentration H.s. = 0.25 mM, C.x. = 0.4 mM.

followed by periodic assessments of motility by the *in vitro* method. The composite of such results (Fig. 3) whereby the responses of all induction treatments and *in vitro* immersions at the highest concentrations for all NFN are averaged, demonstrate for wild type *Pratylenchus vulnus* that the induction treatment provides for increased protection for 45 days. For wild type *X. index* protection increases for approximately two weeks, but decreases thereafter. Induction of stressed *X. index* populations provide slight, if any protection, whereas unstressed *X. index* populations manifest a higher motility before and immediately after induction, but decrease within two weeks to match the response of stressed populations. It must be realized that this is a greatly simplified overview, and the nematode response curves are a function of induction treatment and immersion treatment, and can therefore be diverse (Yamashita & Viglierchio, 1987*b, c*).

One might reasonably assume that stressing a nematode population with a nonfumigant nematicide would result in an adapted nematode population better able to cope physiologically with the nematicide be independent of host properties. Such an assumption would be incorrect (Yamashita & Viglierchio, 1986*a*) as indicated in the summary (Table 2). The effect of host on population structure is evident in the WP ratios, where there are approximately one half as many J2's in grapes as in beans, two and one half times as many J3's, equal numbers of J4's, and a quarter as many adults. It is also evident that the host ratios of different stages vary

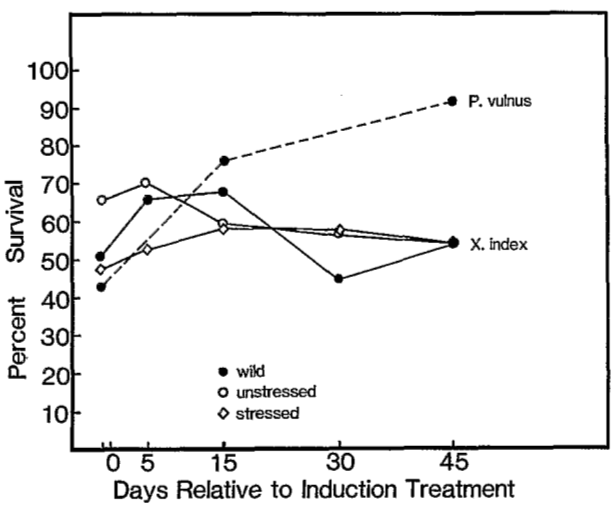

Fig. 3. The activity of *Pratylenchus vulnus* and *Xiphinema index* populations one day prior and subsequent to an induction treatment in terms of apparent survival. Activity reflects movement response to touch after 24 h immersion in 0.6 mM Carbofuran and Oxamyl and 0.16 mM Fenamiphos. For an overview simplification each point represents the composite average of all induction and immersion treatments.

Table 2

Effect of host on stressed and unstressed population structure of *Pratylenchus vulnus* as indicated by grape/bean ratios of different juveniles (J2, J3, J4) and adults (A).

Population	*J2*	*J3*	*J4*	*A*
CSP	2.80	1.90	.47	.32
CUP	1.10	3.10	.74	.24
OSP	2.60	2.10	.72	.42
OUP	7.00	2.40	.31	.15
PUP	2.00	3.10	.58	.32
Aver.*	3.10	2.50	.56	.29
WP	.47	2.40	1.10	.28

CSP = carbofuran stressed population; CUP = carbofuran stressed population removed from stress for two years; OSP = oxamyl stressed population; OUP = oxamyl stressed population removed from stress for two years; PUP = fenamiphos stressed population removed from stress for two years; * Average of stressed and unstressed populations only.

substantially among the preconditioned nematode populations. The composite averages of all preconditioned populations for each stage indicate a high proportion of J2's and J3's and a low proportion of J4's and adults for grapes, with respect to beans. Furthermore, with respect to Wild Population (WP) ratios, the composite average for all preconditioned populations indicate an approximately six-fold greater proportion of J2's, half as many J4's, and approximately the same number of J3's and adults. Whether these differences reflect reproductive fitness, interferences in molting, or other factors in the preconditioned populations is uncertain.

It comes to mind to wonder whether all these laboratory type observations are purely of academic interest or whether they reflect the situation in the field. Fortunately, a recently terminated four year field trial plot on grapes testing NFN was resampled for population levels of the prevalent pests, *X. index* and *M. incognita* (Yamashita & Viglierchio, 1987*d*). In the first year of the trials, the *X. index* population of treatments was decreased to one half the level of controls. However, by the end of the trial of yearly treatments the population levels of *X. index* in carbofuran treated areas were three times the levels from the control plot. Both *X. index* and *M. incognita* from other treatment areas were significantly higher than the numbers taken from the control plot. Moreover, *in vitro* bioassays indicated that nematodes from the treated plots had a higher tolerance to nonfumigant nematicides. These observations were consistent with those of an assortment of different nematodes in field observations with Easter Lilies, other vineyard tests, and turf trials.

It is essential to recognize that the pest nematode problem involves three principle biotic components :

nematodes, microorganisms, host plants. Therefore in pesticide treatments microorganisms would be subject to the same adaptive forces as nematodes. An early experiment (Yamashita, Viglierchio & Schmitt, 1986) using nematode free extracts of carbofuran and fenamiphos stock cultures of *X. index* confirmed the widely held belief that microorganisms could provide protection for the nematode population to subsequent nematicidal treatments. However, a later experiment targeting this phenomenon using nematicide-treated soils from *Heterodera schachtii* nematicide trials demonstrated no such activity, even in tests where the aerobic bacterial component was increased 3 000 fold over normal (Yamashita, Viglierchio & Kuo, 1988*a*). Field microbial degradation of nonfumigant nematicide was demonstrated by radiocarbon studies using treated soils from Costa Rican banana plantations (Anderson & Wybou, 1988). It is evident that soil microorganisms in banana plantations can degrade fenamifos after a lag period during which adaptation takes place (Fig. 4). Fenamifos degradation peaks directly after application. A related pesticide, Isophenphos, is degraded at a five-fold greater rate. The biomass degradative capacity in the absence of pesticide application in certain African fields, virtually disappears after seven months (Fig. 5). The facts suggest that soil microorganisms in general do not degrade soil applied nematicides; however, if those with potential degradative capacity occur, then prolonged pesticide stress encourages their adaptation as well as that of nematodes to provide additional protection to nematodes from nematicide application.

While the emphasis in studies of nematicide stress adaptation has involved plant-parasitic nematodes, the results are in agreement with those obtained with laboratory *in vitro* cultures of *Rhabditis oxycerca* reared on bacterial oatmeal cultures subjected to long-term exposures to Aldicarb and Oxamyl (Kampfe & Wischgoll, 1984). By gradually increasing the stressing concentrations, the nematodes after nine years could tolerate a 400-fold increase in Aldicarb concentration, and a 100-fold increase in Oxamyl. Aldicarb pretreatment increased tolerance to subsequent Oxamyl treatment while Oxamyl pretreated strains showed an increase in susceptibility to Aldicarb; moreover, there were signs of diminished activity in pretreated animals after transfer to nematicide-free media. Reproductive fitness was increased in stressed but not unstressed populations. The microflora of the culture appeared not to be altered by the nematicides. The adaptive changes reported with the bacterial feeding nematode are very similar to those reported for plant-parasitic nematodes.

Practical nematode control technology has fallen on hard times, with few effective and economical practices available. Most probably the late motivation for the types of research just described involves an improved understanding of behavioral relationships to fuel a generation of different and innovative tools to resolve the problem. In this context they have been successful, for they do presage a hopeful future. The potential for the role of chemical agents for nematode control has never been greater, provided that primary attention is paid to the mode of action. For long-term effectiveness, each agent must have a characteristic mode of action, i.e., it must inhibit a different physiological system and yet meet environmental hazard constrictions including mammalian toxicity and pollution. Inasmuch as a primary premise for long-term sustainable nematode control strategy is dependent upon different modes of action, it is essential to secure an understanding of the fundamental target processes. Of the numerous options to gain this information two obvious ones include the determination of enzymic or protein changes, which reflect the mechanisms by which adaptation becomes expressed in nematode populations, and the testing for efficacy of a wide range of biochemical inhibitors evolved by colleagues in related disciplines of the biological sciences. Rudimentary steps have been taken in these directions, but they need to be extended much further (Veech, 1978; Glazer & Orion, 1985; Osman & Viglierchio, 1988; Viglierchio & Wu, 1989).

The potential for adaptability demonstrated to be inherent in pestiferous nematodes is not limited to chemical agents, but is a common integral element of natural adversarial relationships. Plant resistance has long been used as a component of pest management strategies. Experience has demonstrated that multiple gene based resistance is more stable while single gene based resistance whatever the mode of action, is rapidly thwarted by the adaptive processes, which also are often single gene based. To illustrate, the Mi gene, the essentially single gene resistance to major rootknot species, transferred to tomato from the wild type *Lycopersicon peruvianum* has been found to collapse under continued stress (Dalmasso, pers. comm., 1988). Although aggressive field populations have been found occurring naturally in California, Mexico, Africa, and elsewhere, it has been shown that a non-aggressive population inoculated to Mi gene based resistant tomato develops an aggressiveness in three generations that is rapidly lost upon return to normal tomato, however, after some 30 generations on the resistant tomato, the aggressiveness is lost very slowly, if at all.

Biological control methods which are currently receiving appreciable emphasis are also subject to the vagary of adaptive forces. Although adaptability in this area has not been demonstrated as yet with nematodes, entomologists have indicated that lepidopterous larval stages subjected to control measures using *Bacillus thuringiensis* are developing resistance.

In conclusion, the available evidence for nematode control by means of chemical or biological agents and single gene based plant resistance is consistent with the entomologically based empirical five year rule viz., any management tactic under single gene control and used

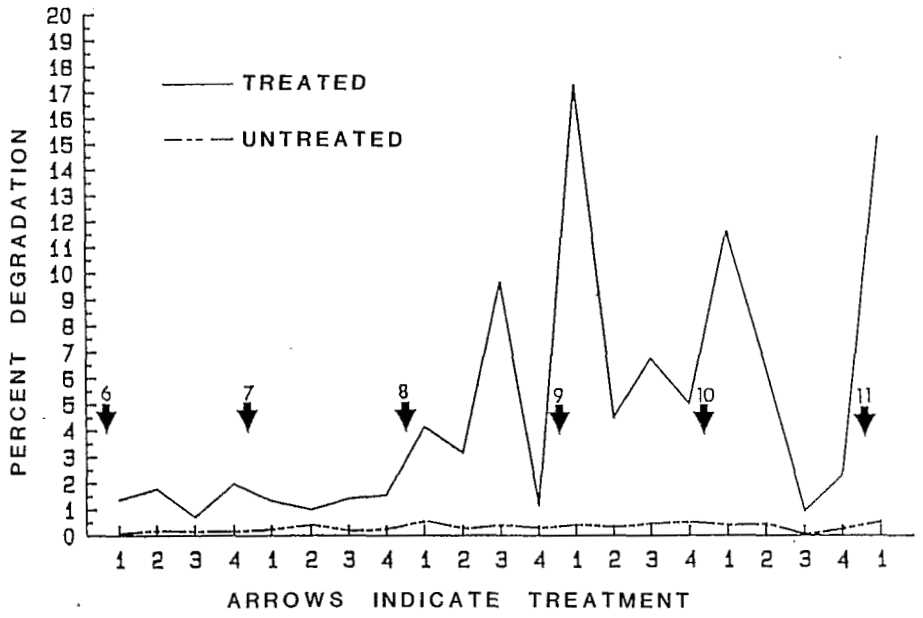

Fig. 4. Degradation of fenamiphos in fenamiphos treated soils from Indiana Tres, Costa Rica. Treatments applied at four month intervals, sampling for degradation analysis done monthly (After Anderson & Wybou, 1988).

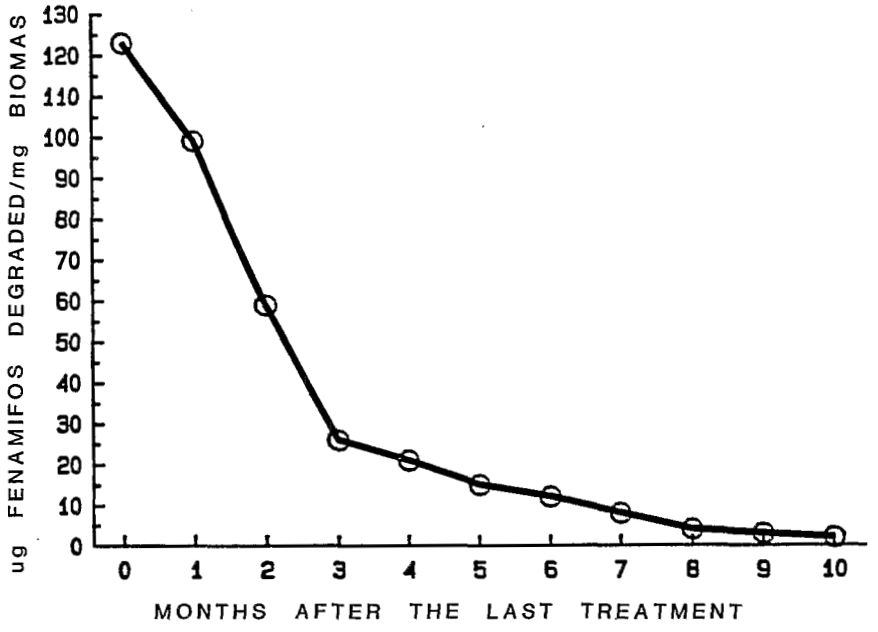

Fig. 5. The recovery time to normalcy after treatment termination of a banana fenamiphos problem soil from the Ivory Coast. Treatment = 40 kg a.i./Ha/yr (After Anderson & Wybou, 1988).

continually is subject to failure within five to fifteen generations of the pest, or usually about five years. Unless the philosophy and the strategy of management or control changes to conform to and work in concert with nature's forces, past mistakes and failures will continue to be repeated.

The expectation that any single agent will be effective in controlling all nematodes is largely fantasy. A revised view requires that different agents, having different spectra of activity based on modes of action, be identified. With the development with some half-dozen agents from any of the three categories discussed, an effective sustainable management protocol can be devised for each nematode problem that avoids adaptation, the development of resistance and a more intractable control situation.

References

ANDERSON, J. P. & WYBOU, A. P. (1988). How do we cope with the biodegradation of non-fumigant nematicides. *Nematropica,* 18 : 1-2 [Abstr.].

Anon. (1986). *Pesticide resistance,* Washington, DC, National Academy Press, 471 p.

BRENNER, S. (1974). The genetics of *Caenorhabditis elegans. Genetics,* 77 : 71-94.

DRUDGE, J. H., SZANTO, J., WYANT, Z. N. & ELAM, T. (1964). Field studies on parasite control in sheep : comparison of thiabendazole, ruelene and phenothiazol. *Amer. J. veter. Res.,* 25 : 1512-1518.

GLAZER, I. & ORION, D. (1985). An induced resistance effect of hydroxyurea on *Meloidogyne javanica*-infected plants. *J. Nematology,* 17 : 21-24.

KAMPFE, L. & WISCHGOLL, S. (1984). Reaction of *Rhabditis oxycerca* after long-term exposure to aldicarb and oxamyl. Part I. General observations. *Nematologica,* 30 : 193-205.

KARTNER, N. & LING, V. (1989). Multi-drug resistance in cancer. *Scientific American,* March : 44-51.

MACDONALD, D. H. (1976). Effects of continual application of aldicarb to greenhouse infested with *Pratylenchus hamatus. J. Nematol.,* 8 : 293-294.

OSMAN, A. & VIGLIERCHIO, D. R. (1988). Efficacy of biologically active agents as nontraditional nematicides for *Meloidogyne javanica. Revue Nematol.,* 11 : 93-98.

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

TRUJILLO-ALVARADO, E. E. (1956). *Studies on the control of nematode diseases of rice with nematicides.* MS Thesis, Univ. Arkansas, Fayetteville.

VEECH, J. A. (1978). The effect of diflubenzuron on egg formation by the rootknot nematode. *J. Nematol.,* 10 : 209-209.

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

VIGLIERCHIO, D. R., BROWN, S. M. & KUO, F. F. (1989). Adaptive responses of *Heterodera schachtii* populations to nematicidal applications of nonfumigant nematicides after stressing with sublethal doses. *Revue Nématol.,* 12 : 133-138.

VIGLIERCHIO, D. R. & WU, F. F. (1989). Selected biological inhibitiors for *Heterodera schachtii* control. *Nematropica,* 19 : 75-79.

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 the stability of behavioral changes in nonfumigant nematicide-stressed populations of *Xiphinema index* following release from nematicidal 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.

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*). 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. (1987*d*). Field resistance to nonfumigant nematicides in *Xiphinema index* and *Meloidogyne incognita. Revue Nématol.,* 10 : 327-332.

YAMASHITA, T. T. & VIGLIERCHIO, D. R. (1988). *In vitro* response to *Criconomella xenoplax* to nonfumigant nematicides. *Revue Nématol.,* 11 : 447-449.

YAMASHITA, T. T., VIGLIERCHIO, D. R. & KUO, F. F. (1988*a*). The role of microbial populations from long-term nonfumigant nematicide treated soils on *Heterodera schachtii* nematicide trials. *Revue Nématol.,* 11 : 351-358.

YAMASHITA, T. T., VIGLIERCHIO, D. R. & KUO, F. F. (1988*b*). Nonfumigant nematicide conditional populations of *Criconemella xenoplax* and their responses to subsequent treatments. *Revue Nématol.,* 11 : 429-325.

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 13 juin 1989.