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An Assessment of Progress toward Microbial Control of Plant-parasitic Nematodes

BRIAN R. KERRY[1]

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In recent years, continuing environmental problems associated with the use of nematicides (47) have introduced a sense of urgency into the search for alternative methods of nematode management. For the past 90 years, research on the biological control of nematodes with microbial agents has been done by a few dedicated nematologists who have worked in small groups with little support.

About 10 years have passed since it was recognized that in some soils nematode multiplication was suppressed by nematophagous fungi. For the first time, biological agents were shown to provide long-term effective control of cyst (29) and root-knot nematodes (45) in the field. However, this control had developed fortuitously in soils and did not result from the release of an agent. At about the same time, a French company produced the first commercial biological control agent for nematodes based on a nematode-trapping fungus (10).

The optimism that resulted from these developments has led to a considerable increase in research effort, but unfortunately too many experiments purporting to demonstrate biological control have been fundamentally flawed and practical difficulties have limited the use of commercial agents. Quite simply, we have not had adequate experimental techniques to determine effectively the potential of most microbial agents. In addition, sufficient resources from government and industry have not been applied to the multidisciplinary approach that is generally recognized as essential for the development of a biological agent. In this article I will discuss the contribution that biological control could make to the management of plant-parasitic nematodes and how to improve our limited understanding so that commercial use of microbial control of nematodes becomes a reality.

APPROACHES FOR RESEARCH

Microbial agents have been considered for four main approaches to control nematodes: 1) exploitation of naturally suppressive soils, 2) soil amendments to encourage the activity of indigenous soil microbes, 3) application of selected strains of bacteria and fungi, and 4) microbial enzymes and toxic metabolites. Considerable difficulties exist in all these approaches, and doubts have been expressed concerning the

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[1] Entomology and Nematology Department, AFRC-IACR, Rothamsted Experimental Station, Harpenden, Hertfordshire, AL5 2JQ, England.

application of single agents for control of nematode pests. Soils that suppress nematode multiplication usually contain a range of natural enemies that attack their host at different stages in its life cycle (25). Each may kill relatively few nematodes, but the combined effects of several enemies may prevent nematode populations from increasing. Because of this and the difficulties in establishing some microbial species in soil, many nematologists consider that the application of a single agent will not provide effective control and that more success will be achieved by enhancing the activities of the indigenous soil microflora. It is premature, however, to make such an assumption; evidence accumulated at Rothamsted and elsewhere indicates that specific agents applied to soil can become established throughout a growing season in sufficient densities to cause significant reductions in nematode populations (8,30). Also, manipulation of the soil microflora to provide control of nematode pests probably cannot be achieved with treatments that are practical on most field, plantation, and orchard crops. The quantities of soil amendments needed to bring about useful changes in the soil microflora are usually well in excess of 5 t/ha and would not be economical on these crops. Commercial development of biological control will probably depend on the selection of a single agent or its products.

Exploitation of naturally suppressive soils: The occurrence of soils that suppress the multiplication of some nematodes has been confirmed. Bacteria, rickettsia-like organisms, and fungi have all been implicated in this natural control of cyst, root-knot, and some ectoparasitic nematodes (26). The development of microbial populations sufficiently dense to control nematodes has occurred only under perennial crops or those grown in monocultures (26); presumably these crops provide a sufficiently stable ecosystem in which an antagonistic microflora can develop and persist (3).

Soils suppressive to the cereal cyst nematode (*Heterodera avenae* Woll.) are particularly widespread in northern Europe and do not appear to be influenced by soil texture (23). Suppression of this nematode appears to take at least 4 years to establish. During this period of establishment, the nematode causes considerable damage and it seems unlikely that growers would be prepared to suffer significant yield losses in the eventual hope of obtaining long-term nematode control. Hence, natural control has tended to develop fortuitously and unobserved. To date, attempts to increase the buildup of the natural antagonistic flora have failed (D. H. Crump, pers. comm.).

Despite the problems noted here, the microflora in suppressive soils could be exploited for management of nematode populations and 1) enable growers to shorten rotations on nematode-infested soil, 2) prolong the useful life of resistant cultivars by parasitizing virulent females, and 3) enhance the long-term effectiveness of nematicides or enable dose rates to be reduced. Therefore, methods that permit rapid identification of such soils are essential. Some techniques have been developed to estimate the numbers of fungi in soil, but few have been used to predict whether a soil would effectively suppress nematode multiplication (11). In general, the methods currently available are too time consuming to be widely used.

Although there may be many different types of natural enemies feeding on a particular pest species in a suppressive soil, it would appear that one or two species are dominant. Thus, in soils that suppressed *H. avenae,* most females and eggs were killed by *Nematophthora gynophila* Kerry & Crump or *Verticillium chlamydosporium* Goddard (24). Although other fungal parasites were also present, they were of minor importance. Similarly, *Dactylella oviparasitica* Stirling & Mankau was considered the major agent in suppressing root-knot nematodes on peach in California (45).

Several workers have shown that microbial agents are more abundant in soils in which nematodes decline than in those where they multiply, but the numbers of propagules required for control have not been determined (9,12,13,46). In cases of

specific suppression an estimation of populations of known parasites may allow prediction of whether nematode populations will decline. For microbes this would necessitate the development of selective media (30,33) or physical methods (12) to determine their abundance in field soils. Nonspecific natural control is difficult to demonstrate experimentally, and methods that would enhance the activity of a range of organisms are unlikely. However, studies on the factors affecting natural nematode control can be of great value in identifying potential biological control agents from suppressive soils and the factors that might affect their efficacy if introduced into soil.

Use of soil amendments to encourage the activity of indigenous soil microbes: A wide range of soil amendments has been tested for their effectiveness in controlling nematode populations (38). In general, it is considered that nematicidal breakdown products, such as ammonia and fatty acids, and the associated enhancement of the indigenous soil microflora are responsible for the control observed (42,44). In general, large quantities of material are required and consequently the use of soil amendments is largely restricted to subsistence agriculture or horticulture in which small areas require treatment. In practice, soil amendments should be waste products that are available locally, so that they are cheap to apply.

Attempts to increase specific groups of organisms selectively have centered on the use of chitin amendments (38). Chitin application has increased microbial activity and chitinase levels in soil, and this has sometimes resulted in significant levels of nematode control. Initially, the degradation of chitin results in the production of ammonia which is nematicidal; subsequent stimulation of the microflora that produce chitinase gives longer lasting control (42). Although there is little doubt that actinomycetes and fungi, in particular, are stimulated by applications of chitin to soil, the exact mechanism by which chitin affects nematodes is unclear. Chitin is present only in the egg shell of nematodes (51) and, although chitinase activity is considered essential for fungal parasitism of eggs (15,17,31), it is difficult to envisage how a general degradation of egg shells would control nematode populations unless eggs were destroyed before they completed their embryonic development or juveniles were released from eggs when no hosts were present. Although frequently assumed, there is little evidence to suggest that chitin increases the activities of nematophagous fungi known to parasitize eggs and females of cyst and root-knot nematodes. Such fungi occur commonly in chitin-amended soil, but their activity may not be increased by applications of chitin. The proportion of cysts colonized by fungi was reduced significantly in chitin-amended, relative to untreated soils (39); applying the equivalent of 45 t chitin/ha merely increased parasitism of eggs of *Meloidogyne arenaria* from 22% in untreated soil to 26% in treated soil (13). Hence, even with selective soil amendments, rates of application are too great to consider practical on a field scale.

The use of green manure crops, widely practiced in some countries, may provide a simple and convenient method of enhancing the activities of nematophagous fungi in soil (41). Such an approach warrants more extensive investigation. Some nematophagous fungi have been grown on waste organic materials, and if these materials are colonized by, or combined with, the agent before they are introduced into soil, the soil amendment effect may be enhanced and provide better levels of control (14,49).

Application of selected strains of bacteria and fungi: The nematologist, having identified a potentially useful disease of a nematode pest and clearly demonstrated its efficacy, requires support from microbiologists and access to skills often residing in industry for the development of mass culturing techniques and for the formulation of suitable propagules into a product. Such a multidisciplinary approach should be established early in the research program because methods of production and type of formulation can have a profound effect

TABLE 1. Analysis of 25 experiments reporting biological control of nematodes using the fungus *Paecilomyces lilacinus*.

Nematode target	Number of experiments	Application rates (t/ha)	Type of test	Observations			
				Adequate checks	Survival or proliferation in soil	Re-isolation from host	Estimated control (%)
Meloidogyne incognita	2	0.2–0.4	Microplot	+	+	+	20–56
	5	0.4–2.5	Microplot	−	−	−	51–90
	1	ND	Microplot	−	−	+	55
	1	6.3–25.0	Pot	+	+	−	0
	1	ND	Pot	+	−	+	84
	2	5.0	Pot	+	−	−	66–96
	1	9.0	Pot	−	−	−	62–74
Meloidogyne arenaria	1	12.5–25.0	Pot	+	+	+	50–70
	2	2.5–12.5	Pot	+	−	−	0–54
Meloidogyne javanica	1	2.2	Microplot	+	+	+	0
	1	0.2–2.5	Pot	−	−	−	52–76
	1	3.8	Pot	−	−	−	55
Globodera rostochiensis	2	1.8	Microplot	−	−	−	41–54
Rotylenchulus reniformis	1	5.0	Pot	+	−	±	44
	2	0.6–5.2	Pot	−	−	−	42–83
Tylenchulus semipenetrans	1	ND	Microplot	−	−	−	75

+ = included/measured, − = not present/measured, ND = no data.

on the growth and survival of a biological agent in soil and, thereby, alter its effectiveness (8).

There has been some criticism of the attitudes of commercial companies toward biological control of nematodes and questions concerning their willingness to provide large-scale financial support. Such research often requires a long-term commitment, however, and too often nematologists have provided too little sound data to inspire confidence in investors. Stirling (43) is critical of much of the research that purports to demonstrate biological control. Too frequently there has been no attempt to re-isolate the agent from its host or to assess whether the agent has survived in soil and might account for the control observed. Also, experiments often have inadequate check treatments, making interpretation of the results difficult. The fungus *Paecilomyces lilacinus* (Thom) Samson is the only agent that has been tested widely in the field; a review of published results (Table 1) indicates that few (ca. 15%) experiments met the basic requirements considered essential to estimate properly the potential of a biological control agent.

The difficulties in satisfying Stirling's (43) criticisms should not be underestimated, however. For example, to determine whether the agent has survived and increased in soil after its application may require selective media to enable isolation from unsterile soils. The development of such media is often laborious; descriptions have been published of only two, *V. chlamydosporium* (30) and *P. lilacinus* (33). The medium for *V. chlamydosporium* took 2 years of research to produce and test in a range of soils under different conditions. In a comparison of three isolates of *V. chlamydosporium* which increased in soil and survived throughout the experiment, only the isolate that was able to colonize the rhizosphere reduced populations of *M. arenaria* (Neal) Chitwood (30). Hence, survival in, and colonization of, soil do not necessarily result in nematode control. Also, assessing the numbers of nematodes killed by the agent can be troublesome because estimations based on a single sampling occasion can be misleading. Nonetheless, it was disappointing to note that more than 50% of the experiments listed in Table 1 had inadequate checks included in their design. In some experiments *P. lilacinus* provided encouraging levels of

control (Table 1), but its efficacy was variable and potential health hazards associated with this fungus are likely to prevent its widespread use (32).

Although the provision of adequate checks is a basic requirement for proper experimentation, their definition has proved difficult in tests involving the application of facultative fungal parasites to soil. These parasites, such as *V. chlamydosporium* and *P. lilacinus*, grow in soil and may colonize the rhizosphere. Unless the fungus produces a resistant resting stage rich in food sources, external sources of energy must be supplied to enable it to overcome competition from the resident soil microflora (27). This energy source is often sterilized and colonized by the fungus before it is added to soil; in this way, fungal agents have been applied on rice, oat, wheat, and millet grains, and bran mixtures. Application rates are often so large that the energy source itself functions as a soil amendment. Hence, applications of the uncolonized energy base and the colonized and autoclaved energy base must be compared with untreated soil and soil treated with the agent alone if true biological control potential is to be estimated. In several experiments, the energy base alone has significantly depressed nematode reproduction and, in some, the presence of the fungus has had little further effect (7). After successful commercial development is achieved, microbial agents will be applied at much lower rates and in forms different from those that have so far been tested. The soil amendment effect of such applications is likely to be minimal, so it is essential in the selection of potential biological control agents to assess the true contribution that the microbial agent itself is making to overall control.

To provide a clear demonstration of biological control requires considerable knowledge of the agent's epidemiology and the development of valid experimental techniques. Such research is very time consuming and beyond the scope of routine screening procedures.

Recently, rhizobacteria have shown potential as biological control agents (5,34). These agents can be applied to seed and may significantly reduce nematode invasion of roots (34). Although some bacteria affected nematode activity in vitro (54), in other tests toxins were not involved; it was suggested that these bacteria affected nematode hatch, attraction toward roots, and host recognition processes, probably through the modification of root exudates (35). Agents that can be applied as seed treatments are strongly favored for use on crops that are grown extensively but, so far, nematode control using rhizobacteria often has been rather variable (5). Nevertheless, protection of the root surface rather than the use of nematophagous organisms provides an exciting new approach for biological control of nematodes. Existing technology could enable the introduction and expression of genes that code for nematicidal products into rhizobacteria to provide novel control methods.

Use of microbial enzymes and toxic metabolites: Little is known of the infection processes and modes of action of nematophagous fungi and bacteria. Exploitation of enzymes or toxins involved in parasitism to develop novel methods of nematode control may not be considered "biological control" in a strict sense, but the subject is attracting increased interest. Research on toxin production in nematophagous fungi has been reviewed (20). Some parasites of nematode eggs are thought to produce metabolites that affect embryonic development and hatching (21). Much of this work was empirical, however, and so far little has been published. The nematicidal properties of avermectins produced by actinomycetes (*Streptomyces* spp.) have stimulated interest in "natural" nematicides, and several companies are screening for toxin production by nematophagous and other soil organisms. Toxins have been found in a range of micro-organisms including the oyster mushroom, *Pleurotus ostreatus* (Jacquin *apud* Fries) Kummer (48), and some strains of the entomophilic bacterium, *Bacillus thuringiensis* Berliner (6).

Three approaches may be considered for using enzymes and metabolites derived from microbes: 1) Mass production could

result in the production of "natural" nematicides, an approach often favored by industry with experience in the development of nematicides, which may give rise to products with additional spectra of activity. 2) Strains of nematophagous microorganisms may be screened and selected for enhanced production of key enzymes or metabolites in the infection process which will enhance their activities as control agents. 3) If these compounds are under simple genetic control, their genes could be transferred to plants or to root-colonizing organisms to disrupt nematode development. Chitinases from egg-parasitic fungi (15) or collagenase from nematode-trapping fungi (40) could be used in this last approach. However, much needs to be learned about infection processes before such exciting possibilities become practical realities.

Selection of Agents

Few micro-organisms have been tested as potential biological control agents, and there is little information on factors that may alter their effectiveness or on methods of production and formulation. The author (26) has reviewed the attributes of effective control agents and the problems involved in their development. Jatala (21) listed 16 characteristics that should be considered in selecting an agent. In general, each potential agent evaluated thus far lacks several "essential" characteristics, but there is little quantitative information on the relative importance of these selection characteristics. For example, can a fungal isolate that is only moderately pathogenic to nematodes, but is rhizosphere competent, be as effective in controlling nematode multiplication as a virulent one that is only a weak rhizosphere colonizer? It is important to consider such questions in the development of selection procedures.

The relative merits of facultative and obligate parasites as potential biological control agents have been discussed (28). Although obligate parasites such as *Pasteuria penetrans* (Thorne) Sayre & Starr are generally considered to be more effective than facultative parasites such as *V. chlamydosporium,* there is no factual evidence to indicate which type of parasite might be best in practice (16,27). Also, obligate parasites have several limitations that may preclude their commercial development. They tend to have limited host ranges, which prevents growth in vitro, or require complex media, which may mean that they have limited markets and will be expensive to produce. Obligate parasites also have no ability to grow and proliferate in soil, so all inoculum required for control must be added to the soil and intimately mixed to ensure contact with the target pest. However, obligate parasites often produce resistant resting structures to ensure their survival when hosts are scarce. These structures usually are resistant to desiccation and enable the organism to be stored and handled more easily than one that can be formulated only as an active mycelium or thin-walled conidia (22). Because facultative parasites grow in soil, their efficacy can be affected by soil conditions and application rates may have to be increased to overcome detrimental effects. For example, *V. chlamydosporium* occurs naturally in a wide range of soil types, but some isolates establish much more readily in organic than in mineral soils (28). Hence, greater rates of application might be required to control nematodes in mineral than in organic soils.

At Rothamsted, research has concentrated on the use of *V. chlamydosporium* as a biological control agent for cyst and root-knot nematodes. Many factors influence the relationship between the amount of fungus applied to soil, the extent of rhizosphere colonization, and the level of control achieved. A thorough understanding of these interactions should lead to improved methods of control. Application rate (30), method of application (27,30), soil texture (28), and fungal isolate (28,30) affect the survival and proliferation of *V. chlamydosporium* in soil. Also, the plant host (30), timing of infection (27), nematode species, and density may influence the proportion of nematodes infected. Other factors not yet studied, such as rhizosphere competition, soil temperature, rate of nematode development and reproduction, are also likely

to be important factors affecting the efficacy of *V. chlamydosporium* as a biological control agent. In our experience, rhizosphere competence is essential for control, and the amount of fungus ultimately produced is affected by the application rate, crop species, and nematode damage. Root damage by nematodes increased the development of *V. chlamydosporium* on tomato roots (30). Presumably, more nutrients that supported fungal growth in the rhizosphere leaked from nematode-damaged roots than from those that were healthy. However, if rates of application are unrealistically large, isolates that do not colonize the rhizosphere still may be able to control nematodes.

Verticillium chlamydosporium can influence cyst nematode multiplication in several ways (Table 2). Hence, it may prove difficult to relate nematode multiplication to the numbers of eggs colonized at the end of the experiment. It may be necessary to monitor fungal infection on several occasions during the maturation of the female nematode. Control of *Heterodera schachtii* Schmidt by different isolates of *V. chlamydosporium* was related to the proportion of young females infected but not to the numbers of cysts colonized (27); infection resulted in few eggs being produced and many of those were parasitized (28). Thus, it may be difficult to satisfy Stirling's criticisms (43) since control may be related to the cumulative effect of several aspects of infection. Routine screening of potential agents to check all of these avenues of infection would therefore be very time consuming.

Screening procedures: Potential biological control agents are more likely to be found in nematode-suppressive soils in areas where the target pest is indigenous. If it is known that a particular stage of a pest species is vulnerable, then it makes sense to isolate organisms from that stage. Methods for isolating organisms from diseased nematodes have been reviewed (24). Once isolated in pure culture, tests can begin to determine the potential of the organism as a biological control agent. Since different isolates of most species so far tested vary greatly in a number of important characteristics, there is a need to screen relatively large numbers and to develop simple methods of assessment. Potentially useful isolates that are identified should then be tested in more detail to determine factors affecting efficacy.

TABLE 2. Effect of *Verticillium chlamydosporium* on the development and fecundity of females of the beet cyst nematode, *Heterodera schachtii*.

	Symptom occurrence†	
	V. chlamydosporium	Untreated
Females/plant (no.)	21	106
Colonized females (%)	29	12
Eggs/female (no.)	32	401
Infected eggs (%)	79	6
Length of female (μm)	550	830
Breadth of female (μm)	375	630

From Kerry (23).

† Comparative data on symptom occurrence in *H. schachtii* infected by a pathogenic isolate of *V. chlamydosporium* applied to compost vs. untreated controls. Means of five replicates.

The lack of information on epidemiology, mode of action, and survival in soil has led to the development of screening methods based on intuition rather than fact. Applying an agent to soil in a pot test and then measuring nematode reproduction and plant damage gives only limited information on its biological control potential. Lack of control could result from inappropriate application methods that failed to establish the organism in soil. For example, *Hirsutella rhosilliensis* Minter & Brady is infective only when its adhesive spores remain attached to their phialides on the mycelium (19), and applications of aqueous suspensions of conidia are not infective unless they first produce more spores in soil.

A simple screen on agar which ensured good contact between agent and nematode target (18) detected considerable differences in virulence to nematode eggs among isolates of *V. chlamydosporium* (Table 3). Levels of infection tended to be low in such tests because they used mature eggs, which are less susceptible to parasitism than those that do not contain second-stage juveniles (18). Immature eggs are more difficult to obtain in the large numbers required for

TABLE 3. Infection of eggs of three species of nematodes used in an in vitro screen of 103 isolates of *Verticillium chlamydosporium*.

Nematode target	Infection by fungal isolates: Mean (%)†	± S.E.	Range of activity (%)‡
Heterodera avenae	28	1.1	10–57
Globodera rostochiensis	21	0.8	4–49
Meloidogyne incognita	29	1.1	4–63

† Mean of five replicates.
‡ Range of activity of best and poorest isolate against nematode target.

screening. There was little difference in susceptibility of the three nematode species (Table 3), but no fungal isolate infected all hosts equally well. As expected, performance on agar did not necessarily relate to efficacy in soil. However, those isolates that performed badly in the agar test never showed activity in soil and so could be discarded. Since pot tests are time consuming and labor intensive, there is a need for simple tests, such as performance on agar, to reduce the number of isolates for further selection. Results from such tests must be treated with caution, however. Similarly, simple in vitro tests for rhizobacteria enabled 5,000 isolates, collected at random from crop plant rhizospheres, to be screened; only 1% showed some activity and, of these, only 20% showed activity in a soil test (15). Screening procedures must be simple when potentially useful isolates are scarce, or the initial isolation procedures must be more selective to increase the proportion of potentially useful isolates.

Pasteuria penetrans, P. lilacinus, V. chlamydosporium, and *H. rhosilliensis* are being studied in some detail in a number of laboratories. Although these four organisms may not represent those with the most potential as biological control agents, research on them should lead to the development of suitable techniques and increase our understanding of the key factors involved in the microbial control of nematodes. The development of mathematical models (36,37) of these interactions could be particularly instructive; the need for quantitative data would impart a discipline on the research that hitherto has often been lacking.

FUTURE PROSPECTS

In recent years, a number of factors have come together that should ensure continued support for research on biological control. The need to replace current nematicides, political pressures for pest management programs that do not depend on pesticides, and demonstrations that selected agents might provide effective control have all contributed to a change in attitudes toward research on biological control. Most statements on future research strategies for agriculture stress the need for increased resources for work on the natural enemies of pests, diseases, and weeds. Several chemical companies have established biological control research programs and have identified nematodes and soil-borne diseases as suitable targets because chemical methods of control are either lacking or increasingly unacceptable. Support for biological control is likely to be sustained even if a new generation of effective and safe nematicides are developed.

Biological control agents should not be seen as replacements for nematicides, since they are unlikely to be as effective or fast acting. To maintain efficient nematode management, biological methods would have to be integrated with other methods, such as solarization (50), plant resistance (30), and low rates of nematicides (4), or applied to relatively small nematode infestations as preventative measures (9,30). These approaches would require more expert supervision to determine the right conditions and time for application of the agent than is needed for chemical control. However, pressure for change in crop protection methods may lead to such limitations becoming accepted. Many applied nematologists currently fear that more nematicides will be withdrawn from the market before there are suitable alternative methods of control.

Although food shortages are not as acute in 1990 as they were in the 1960s, continuing population pressure ensures the need

to increase average yields of most major crops (1). Inferior methods of nematode control are no more acceptable now than they were then. The challenge is to provide effective and environmentally benign control methods. Since results may depend on methods of formulation and mass production, research will need support from industry long before efficacy in large-scale field trials has been demonstrated. Field tests should be done as soon as possible, particularly if selective media are available to monitor the agent after its release, because we still know very little about the key factors that affect the establishment and effectiveness of biological control agents in the field. It is important to identify a suitable nematode target, preferably one that is a pest in protected or horticultural crops in which it may be possible to control conditions to favor the released organism.

Nematode control on arable crops may be commercially more attractive than in the small markets in horticulture, but arable crops present greater problems for biological agents because they are grown over large areas and are of relatively low value. More information could be obtained from practical experience of the use of an agent for nematodes in a confined situation. In the UK, the Agricultural Genetics Company initially restricted use of their entomophilic nematode product, "Nemasys" (based on *Steinernema bibionsis* Bovien), to control of vine weevils on cyclamen (*Cyclamen persicum* Mill.). Because production was concentrated on only 30 growers, careful monitoring of product use was possible and valuable information was obtained on factors affecting efficacy. Thus, development of the product was able to continue without loss of confidence by the growers, representing a successful approach that might be considered for the development of other biological agents.

Fundamental research on epidemiology, biology, and mode of action, all of which are essential to underpin the development of selected biological control agents, must be supported by government. Similarly, research on nematode-suppressive soils and natural control is unlikely to be supported by commerce and thus would also require central funding. Both commercial and government sources of funding may support much-needed surveys for new agents. Despite declared interest, the levels of funding for research on biological control has not greatly increased in recent years. Clear demonstrations of efficacy in the field may be required if this situation is to change substantially (44).

It has been stated that to develop effective biological control we need to understand the structure, development, dynamics, and regulation of nematode communities on roots (2). To develop such an understanding will require the resources of a well-equipped multidisciplinary team for many years. Practical needs and political pressures lead to the conclusion that whatever the merits or demerits of single-agent release, such an approach provides a useful way of perturbing the balance of a nematode population and studying the interactions with the biological control agent. Hence, with proper quantitative techniques for estimating populations of both nematode and agent, information concerning factors affecting control may be obtained more efficiently than by pursuing a more holistic approach.

Experience to date should have taught us that predictable biological control of any nematode pest will not be achieved easily and will require careful and detailed research. Given that commitment, the opportunity has probably never been better for research on, and development of, biological control methods and their eventual incorporation in nematode management programs.

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