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Biological Control of Plant-Parasitic Nematodes:

Building Coherence between Microbial Ecology and Molecular Mechanisms

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Progress in Biological Control

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Progress in Biological Control

Series Preface

Biological control of pests, weeds, and plant and animal diseases utilising their natural antagonists is a well-established and rapidly evolving field of science. Despite its stunning successes world-wide and a steadily growing number of applications, biological control has remained grossly underexploited. Its untapped potential, however, represents the best hope to providing lasting, environmentally sound, and socially acceptable pest management. Such techniques are urgently needed for the control of an increasing number of problem pests affecting agriculture and forestry, and to suppress invasive organisms which threaten natural habitats and global biodiversity.

Based on the positive features of biological control, such as its target specificity and the lack of negative impacts on humans, it is the prime candidate in the search for reducing dependency on chemical pesticides. Replacement of chemical control by biological control – even partially as in many IPM programs – has important positive but so far neglected socio-economic, humanitarian, environmental and ethical implications. Change from chemical to biological control substantially contributes to the conservation of natural resources, and results in a considerable reduction of environmental pollution. It eliminates human exposure to toxic pesticides, improves sustainability of production systems, and enhances biodiversity. Public demand for finding solutions based on biological control is the main driving force in the increasing utilisation of natural enemies for controlling noxious organisms.

This book series is intended to accelerate these developments through exploring the progress made within the various aspects of biological control, and via documenting these advances to the benefit of fellow scientists, students, public officials, policy-makers, and the public at large. Each of the books in this series is expected to provide a comprehensive, authoritative synthesis of the topic, likely to stand the test of time.

Heikki M.T. Hokkanen, Series Editor

Preface

The need for alternative management systems for the control of plant-parasitic nematodes has increased dramatically over the last decade, mainly because of the banning of the most important nematicides. Therefore, biological control of phytonematodes has received an enhanced impetus and several attempts in the industrial/ commercial sector as well as in academia, have been made to fulfill this need. The last relevant handbook on this treatise was published in 1991 and since then there has been no specific volume addressing this important topic. This book was written at a time when molecular biology as well as different 'omic' approaches, were just beginning to encroach on the subject area but were not included. Therefore, the progress that has been made in biotechnology and the new tools available for research have augmented new perspectives that help in our understanding, in areas as diverse as as aspects of mode-of-action through population dynamics to knowledge about formulation and application techniques, which have so far not been covered by any other volume.

The offered volume intends to review the biological control theme from several prospects: (1) Various ecological aspects such as: suppressive soils, organic amendments, issues related to the farming system both at present and in the future together with the role of nematodes in soil food webs, that covers application, conservation and enhancement of indigenous and introduced antagonists (Chaps. 1, 2 and 11); (2) *Caenorhabditis elegans* as a model and lessons from other natural systems (Chap. 3); (3) Exploiting advanced genomic tools to promote the understanding of biocontrol processes and thereafter helping to improve specific biological control agents (Chaps. 3, 4, 6 and 7); (4) Interaction between the plant host, nematodes' surface and microorganisms: the role of the nematode surface-coat in interactions with their host-plant and their surrounding bacteria and fungi (Chap. 5), emphasizing on the biochemical, molecular and genomic interactions of nematodes with nematode-trapping fungi (Chap. 6), and understanding the mode-of-action of various biocontrol systems such as the eggs- and cyst-parasite *Pochonia chlamydosporia* (Chap. 7) and *Trichoderma* spp. (Chap. 8). (5) Candidates for biocontrol - microorganism's applicative as well as commercial state of the art (nematode-trapping fungi, endophytes fungi, *Pochonia chlamydosporia*, *Trichoderma* sp., or *Pasteuria penetrans* (Chap. 4, Chaps. 6–10); and (6) Extrapolation of the wide knowledge existed in another systems for understanding biocontrol processes (Chap. 9).

This volume comprises a wide spectrum of topics and ideas relevant not only to biological control of plant-parasitic nematodes, but also to generic aspects of host- parasite interactions that can be used by scientists with little knowledge or experience with phytonematodes.

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Chapter 1
Biological Control of Plant-Parasitic Nematodes: An Ecological Perspective, a Review of Progress and Opportunities for Further Research

Graham R. Stirling

Abstract Plant-parasitic nematodes are important pests, causing billions of dollars damage to the world's food and fibre crops. However, from an ecological perspective, this group of nematodes is simply one component in a vast array of organisms that live in soil. All these organisms interact with nematodes and with each other, and during that process, contribute to regulatory mechanisms that maintain the stability of the soil food-web. Populations of individual species do not increase indefinitely but are subject to a constant series of checks and balances, which more or less stabilises their population densities. Thus, biological control is a normal part of a properly functioning soil ecosystem, with plant-parasitic nematodes only becoming pests when they are no longer constrained by the biological buffering mechanisms that normally keep them in check. This chapter therefore focuses on approaches that can be used to restore, maintain or enhance the natural nematode-suppressive mechanisms that should operate in all agricultural soils. The positive impact of organic matter and the negative effects of tillage, biocides, fertilisers and other management practices on suppressiveness are discussed, together with examples of suppression due to host-specific natural enemies. The problems associated with replacing soil fumigants and nematicides with biological alternatives, and the ecological issues likely to affect the efficacy of such products, are also considered.

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1.1   Introduction

The relatively stable behaviour of animal populations in natural environments should serve as a constant reminder that in nature, all organisms are subject to a constant series of checks and balances. Populations of individual species do not increase indefinitely but are constrained by the physical environment and by the community of organisms within which they co-exist. Cyclic changes in populations will occur, but provided there is no major change in the physical or biotic environment, populations will fluctuate between certain upper and lower limits. This phenomenon, commonly referred to as 'biological balance' or the 'balance of nature', more or less stabilises animal population densities and applies to all organisms, including plant-parasitic nematodes. The action of soil organisms in maintaining nematode population densities at lower average levels than would occur in their absence is generally termed 'biological control'.

These words, which were included on the first page of my book on biological control of nematodes (Stirling 1991) define the general area of biological control, indicate that it operates wherever nematodes occur, and remind us that plant-parasitic nematodes only reach unacceptably high population densities (i.e. become pests of economic concern) when they are no longer constrained by the biological mechanisms that normally keep them in check. Phrases such as 'the balance of nature' also provide a focus for this chapter, because the aim is to discuss biological control of nematodes within an ecological framework. Thus the chapter begins with a discussion of the soil environment and the regulatory forces that operate within the soil food web and then considers how these natural regulatory mechanisms can be exploited in various farming systems to improve the level of nematode control achievable by biological means.

1.2   Fundamentals of Soil Ecology

It is only in the last few decades that ecologists have undertaken detailed studies of belowground soil processes, and this has led to a better understanding of the nature of the soil environment and the complex biological communities that live in soil. Bacteria and fungi have always been recognised as the most numerically abundant members of the soil biota, but culture-independent molecular tools are now indicating that they are far more numerous and diverse than previously thought (Coleman 2008; Buée et al. 2009a, b). Our knowledge of the feeding habits of the microfauna (e.g. protozoa), mesofauna (e.g. rotifers, nematodes, tardigrades, collembolans, mites and enchytraeids) and macrofauna (e.g. earthworms, termites and millipedes) is also improving, and this is giving us a better insight into the numerous biotic interactions that occur within the soil environment, and how these interactions influence major ecosystem processes such as organic matter turnover and nutrient cycling. These issues are only covered briefly here, but further information is available in several comprehensive textbooks in soil microbiology (e.g. Tate 2000;

Davet 2004; Sylvia et al. 2005; Paul 2007; van Elsas et al. 2007) and in recent books on soil biology and ecology (e.g. Wardle 2002; Coleman and Crossley 2003; Bardgett 2005).

1.2.1   The Soil Food Web

The reason for interest in biological control of nematodes is that some plant-feeding nematodes are important pests, causing billions of dollars damage to the world's food and fibre crops. However, from an ecological perspective, this group of nematodes is simply one component of a large community of organisms that make up what is known as the soil food web. This community is sustained by the photosynthetic activity of plants, its food supply coming from roots, root exudates and plant-derived materials that either accumulate on the soil surface or become available when roots die. The primary consumers within the food web are bacteria, fungi, plant-feeding nematodes and root-grazing insects that feed directly on living plant roots, and the bacteria and fungi that decompose detritus. However, bacteria and fungi are by far the most important component of the soil food-web: they comprise most of the living biomass in soil and are primarily responsible for breaking down and mineralising organic compounds from plant tissue.

The resources transferred from plants and detritus to primary consumers do not remain locked up for very long because these organisms soon become food and energy sources for secondary consumers. Thus bacteria are consumed by nematodes and protozoa, fungal hyphae are pierced by stylet-bearing nematodes and then plant-feeding and free-living nematodes are parasitised by fungi or eaten by predators. These secondary consumers are eventually utilised by organisms at higher levels in the soil food web, while nutrients that are defecated, excreted or contained in dead bodies are also a resource for other organisms. Thus the soil food-web contains a complex array of interacting organisms with numerous pathways that transfer energy from producers (plants) to primary and secondary consumers. Since some of the resources available to the food web are lost at each trophic interchange due to respiration, detrital food chains do not continue indefinitely. They are generally limited in length to about five members (Coleman and Crossley 2003).

1.2.2   Functions of the Soil Food Web

The two most important functions of the soil food-web are to decompose plant material that enters the soil as litter and dead roots, and to mineralise the nutrients contained within that organic matter so that they can be re-used by plants. The decomposition process is mainly the result of microbial activity, but the soil fauna plays a role by fragmenting and ingesting organic matter, thereby increasing the surface area available for microbial colonisation. As plant material is decomposed,

elements are converted from organic to inorganic forms that can be taken up by plants or used by microbes. This process is of critical importance in natural ecosystems (e.g. forests and grasslands), as almost all the nutrients required to sustain primary productivity are derived from mineralisation of soil humus and indigenous biomass. The soil food web also has many other important functions, as it regulates populations of plant pests and pathogens (discussed in the following section), immobilises nutrients within microbial biomass, sequesters carbon, detoxifies pollutants and stabilises soil aggregates.

1.2.3   Biotic Interactions Within the Soil Food-Web

The soil food-web contains huge populations of innumerable species and these populations are continually interacting with each other. These interactions become more complex as the diversity within the soil food-web increases, with multiple forces exerting pressures that prevent the uncontrolled proliferation of particular populations. Interactions between populations therefore have the effect of stabilising the community that makes up the food-web.

Given the complexity of the soil food-web, it is not surprising that populations interact in many different ways. Davet (2004) gives examples of the types of interaction that can occur, and most are relevant to a discussion of biological control.

Antibiosis is the inhibition of one organism by the metabolic product of another. It usually involves interactions where the adversary is killed or inhibited but is not consumed. The metabolic products (usually soluble or volatile antibiotics) are produced in such small quantities by bacteria or fungi that it is difficult to prove conclusively that they are present in the natural environment. Nevertheless, they are known to play a role in interactions between various plant pathogens and the soil biota, with one well-studied example being inhibition of the take-all pathogen *Gaeumannomyces graminis* var. *tritici* by two antibiotics (2,4-diacetylphloroglucinol and phenazine-1-carboxylic acid) produced by fluorescent pseudomonads on wheat roots (Weller et al. 2002).

Lysis is similar to antibiosis in that its effects are manifested at a distance from the organism responsible for lytic activity, but differs in that the adversary is exploited. It occurs when an organism produces extracellular enzymes (e.g. chitinases, cellulases and glucanases) that digest the cell wall or cuticle of another organism. Sometimes the process is accompanied by the production of toxins that immobilise or kill the prey. Bacteria, and more particularly actinobacteria, are significant producers of lytic enzymes and toxins, and important agents in the lysis of fungi.

Predation is generally characterised by the consumption or assimilation of one organism (the prey) by a larger organism (the predator). It requires intimate contact between the two organisms and usually involves an active search for the prey by the predator. Protozoans, nematodes and microarthropods all have the capacity to consume other soil organisms, some feeding indiscriminately on a wide range of

organisms and others having quite specific food preferences. With respect to nematodes, predators of bacteria and fungi can be differentiated from predators of organisms further along the food chain by referring to the latter as 'top predators'.

Parasitism occurs when an organism (the parasite) lives in or on another organism (the host) and obtains all or part of its nutritional resources from that host. Bacteria and viruses are known to parasitise some soil organisms (e.g. protozoans and nematodes), but fungi are probably the most important parasitic organisms in soil. Numerous fungal parasites of arthropods and nematodes are known, and mycoparasitism (parasitism of one fungus by another) is also commonly observed.

Competition between organisms occurs when the amount of an essential substrate or nutrient is insufficient to satisfy the needs of both organisms. The organism most adept at accessing the limiting element, making it inaccessible to others or eliminating those trying to obtain it, will prosper relative to its competitors. Competition is a universal phenomenon within the soil food web, but becomes particularly intense when organisms in the same ecological niche are attempting to access the same scarce resource.

The word **antagonism** is often used instead of antibiosis to describe the situation where one organism inhibits another through antibiotic production. However, the term is used in a more general sense in this chapter to cover all situations where one organism (the pest) is detrimentally affected by the actions of other organisms. Such a definition is commonly used in the literature on biological pest control, as it is useful for describing the general suppressive effects of an organism on a pest, regardless of whether the antagonist is acting through parasitism, predation, antibiosis, competition or some other process.

Although the above mechanisms depict the types of interaction that occur between organisms in the soil food web, outcomes from these interactions are not easy to predict. Environmental factors have marked effects on relationships between organisms, while the interactions between two organisms will be modified by the introduction of a third organism. Thus the structure of a microbial community is the result of environmental effects and multiple interactions that are often quite difficult to comprehend.

1.2.4   Biotic Interactions in the Root Zone

The principal means by which plant roots impact on soil food webs is through the quality and quantity of organic matter that they return to soil. These carbon inputs are derived from fine roots (which have a relatively short life span and rapid turnover times), from cells that slough off as roots move through the soil, and from root exudates. Exfoliation and exudation from roots are particularly important processes because they contribute sugars, amino acids, mucilage and other materials that are high quality nutrient sources for rhizosphere microorganisms. Thus the area in the immediate vicinity of roots is a zone of intense biological activity and complexity

(Buée et al. 2009a). Since herbivores such as arthropods, plant-parasitic nematodes and pathogenic fungi also live in this zone, their activities are most likely to be influenced by organisms that are able to establish and maintain themselves in this extremely competitive ecological niche.

The surface of the root (often referred to as the rhizoplane) is a particularly important niche for soil microorganisms. Some of these organisms thrive in regions where exudation is most intense and protective mucilage is thickest, others survive saprophytically on senescent epidermal and cortical cells, and others are endophytes, colonising root cortical tissue and living in a symbiotic association with the plant. Mycorrhizal fungi are a well-known example of the latter association, as they receive carbon substrates from the plant and provide fungal-acquired nutrients to the plant. Since ramifying mycelial filaments affect soil structure and the mycorrhizal colonisation process improves plant growth, alters root morphology, changes exudation patterns and provides some protection against root pathogens, mycorrhizae influence the biotic interactions that occur in and near roots. Other symbiotic associations also add complexity to the soil-root interface. Examples include rhizobia and other bacteria that fix nitrogen in nodules on plant roots; plant growth promoting rhizobacteria that enhance seed germination and plant growth; and endophytic fungi that deter pests from feeding on plants or improve the plant's capacity to adapt to stress conditions.

1.3   Soil Ecology and Biological Control

The preceding discussion demonstrates that plant-parasitic nematodes cannot be considered in isolation from other components of the soil biological community. Their root-feeding habit brings them into contact with a vast number of root and rhizosphere-associated microorganisms and they also interact with numerous organisms in the detritus food web (Fig. 1.1). Additionally, the activities of plant-parasitic nematodes and other soil organisms are influenced, directly and indirectly, by various soil physical and chemical properties and by environmental factors such as temperature and moisture. These ecological realities must be recognised in any discussion of biological control.

One reason for opening this chapter with a general discussion of soil biology and ecology is to make the point that biological control is a normal part of a properly functioning soil ecosystem. Numerous soil organisms interact with nematodes and with each other and in that process they contribute to the regulatory mechanisms that maintain the stability of the soil food-web. Since plant-feeding nematodes become pests when these biological buffering processes are inadequate, biological control should be thought of as maintaining, restoring or enhancing the natural suppressive mechanisms that exist in all soils. Given that it may take months or years to arrive at a new 'balance' of interactions, the difficulties involved in shifting a stabilised system to a new equilibrium should not be underestimated.

Although most nematologists have some understanding of soil ecology, many fail to view biological control from an ecological perspective. Instead, biological
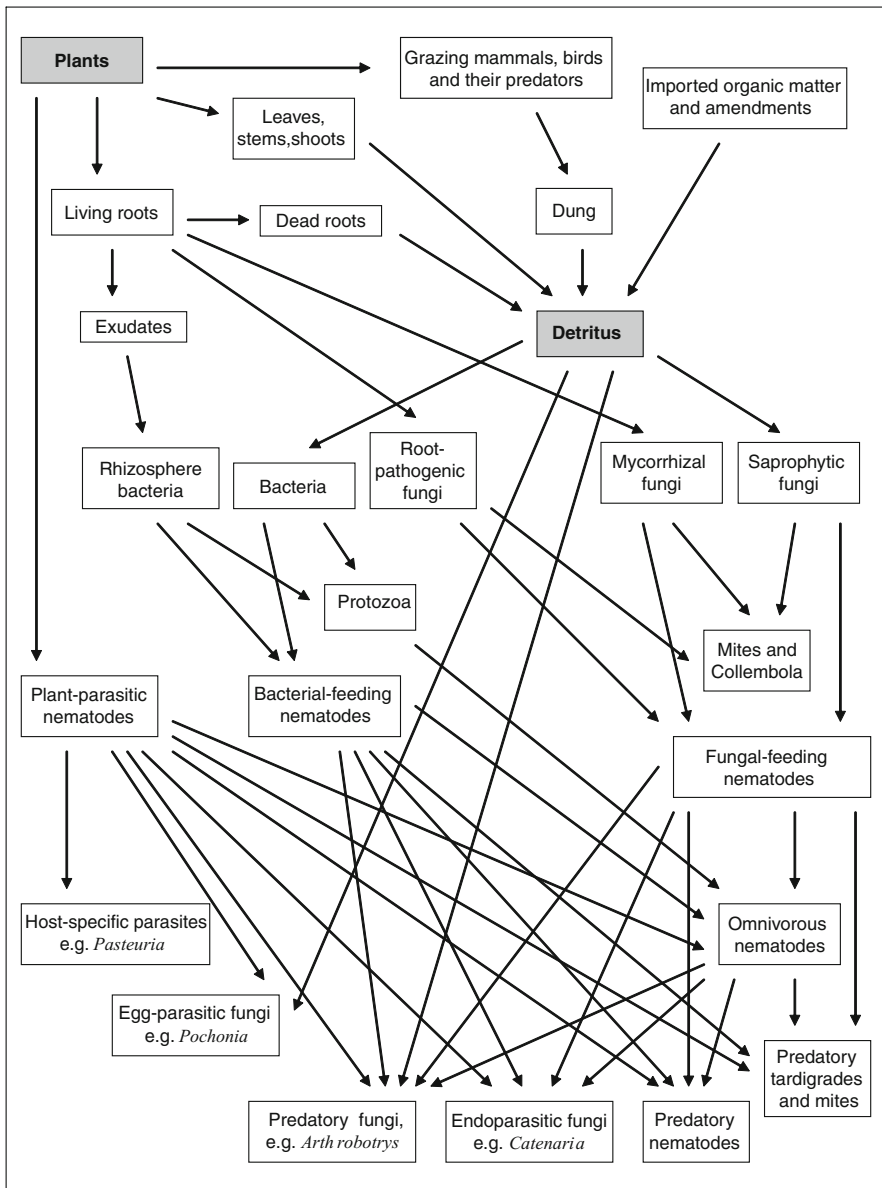
Fig. 1.1 Representation of a soil food web, showing the main interactions between plant-parasitic nematodes, some other primary consumers, and the detrital food web

control is thought of, in relatively simplistic terms, as the introduction of beneficial organisms to control a pest. Most farmers are no different. Having depended on soil fumigants and nematicides for many years, they consider that biological control is about replacing relatively toxic chemicals with safe biological products. Thus there is a common perception amongst both professionals and growers that given time

and an appropriate amount of research, we will eventually be able to reduce nematode populations to non-damaging levels by adding a biological pesticide to soil. I suggest that given the likely cost of producing and distributing such products and the ecological complexity of soil, this approach is unlikely to be successful, except perhaps in specific and quite limited circumstances (discussed later). This chapter, therefore, focuses on other approaches to biological control.

1.3.1   What Is Biological Control?

As pointed out by Stirling (1991), there are a wide range of opinions on what constitutes biological control, with plant pathologists and entomologists often differing on the meaning of the term. The definition used by Baker and Cook (1974) has been adopted here because of its relevance to all plant pathogens, including plant-parasitic nematodes. Thus biological control is considered to:

- Involve the action of one or more organisms
- Result in a reduction in nematode populations or the capacity of nematodes to feed on the plant or cause damage
- Be accomplished in a number of possible ways:

  - Naturally
  - By manipulating the environment, the host plant or the soil food web
  - By introducing one or more antagonists

As mentioned previously, the last-mentioned approach has tended to dominate biological control thinking for many years, whereas the attraction of the above definition is that it takes a more holistic view of the topic. Mass introduction of fungal and bacterial parasites of nematodes is still an option, but is only one of many possible ways of maintaining nematode populations below damaging levels through the action of parasites, predators and other antagonists. Such a definition encourages us to think about how a suite of organisms might act together to regulate a nematode population, to consider why natural suppressive forces are effective in one environment but not another, and to consider how a farming system might be modified to enhance the level of biological control that will already be occurring.

1.4   Suppressive Soils

Soilborne pathogens debilitate roots or cause wilt, root-rot and damping-off diseases in most of the world's crops. Although these pathogens are widely distributed, there are situations where disease severity is lower than expected, given the prevailing environment and the level of disease in surrounding areas. In some of these cases, the indigenous microflora is the reason plants are effectively protected from the pathogen, a phenomenon that is known as disease-suppression. Books by Baker and

Cook (1974), Cook and Baker (1983), Hornby (1990) and Stirling (1991) summarise much of the early work in this area and discuss many examples of suppressiveness to nematodes and other soilborne pathogens.

Two types of disease suppressiveness can occur in agricultural soils. The most common (often referred to as 'general' or 'non-specific' suppressiveness) is found in all soils and provides varying degrees of biological buffering against most soilborne pests and pathogens. Since the level of suppressive activity is broadly related to total soil microbial biomass and is therefore enhanced by practices that conserve or enhance soil organic matter, the term 'organic matter-mediated general suppression' is also commonly used (Hoitink and Boehm 1999; Stone et al. 2004). This type of suppression can be removed by sterilising the soil and is due to the combined effects of numerous soil organisms.

A second form of suppression (usually known as 'specific' suppressiveness) is also eliminated by sterilisation and other biocidal treatments but differs from general suppressiveness in that it results from the action of a limited number of antagonists. This type of suppression relies on the activity of relatively host-specific pathogens and can be transferred by adding small amounts of the suppressive soil to a conducive soil (Westphal 2005). Since specific suppression operates against a background of general suppressiveness (Cook and Baker 1983), the actual level of suppressiveness in a soil will depend on the combined effects of both forms of suppression.

1.4.1   Broad-Spectrum, Organic Matter-Mediated Suppression

The role of organic matter in enhancing suppression of soilborne diseases caused by fungi, Oomycetes, bacteria and nematodes has been known for many years and there are now well-documented examples in many quite different agricultural systems. These include suppression of *Pythium* in Mexican fields following the application of large quantities of organic matter over many years (Lumsden et al. 1987); broad-spectrum control of *Pythium*, *Phytophthora* and *Rhizoctonia* in peat and compost-based soilless container media (Hoitink and Boehm 1999); the use of cover crops, organic amendments and mulches to suppress *Phytophthora* root rot of avocado in Australia (Broadbent and Baker 1974; Malajczuk 1983; You and Sivasithamparan 1994, 1995); suppression of the same disease with eucalyptus mulch in California, USA (Downer et al. 2001); the management of a fungal, bacterial and nematode-induced root disease complex of potato in Canada with chicken, swine and cattle manures (Conn and Lazarovits 1999; Lazarovits et al. 1999, 2001), and the use of crop residues, animal manures and organic waste materials to reduce damage caused by plant-parasitic nematodes (reviewed by Muller and Gooch 1982; Stirling 1991; Akhtar and Malik 2000; Oka 2010).

It is obvious from the above examples that a wide range of types and sources of organic matter can be used to enhance suppressiveness and that they are effective in many different situations. However, studies (summarised by Hoitink and Boehm

1999 and Stone et al. 2004) in relatively simple nursery potting media have given us a much better understanding of the mechanisms involved. Suppression is generated soon after an amendment is added to soil and is associated with the activity of indigenous microorganisms that colonise organic material during the decomposition process. Development of suppression is associated with high levels of microbial activity, with many studies showing that the rate of hydrolysis of fluorescein diacetate (FDA) is a relatively good indicator of suppressiveness. Since microbial activity must remain high to maintain suppressiveness, the quantity and quality of the organic inputs have a major impact on the duration of suppressiveness. The labile constituents of organic matter (e.g. sugars, proteins and hemicelluloses) are degraded relatively quickly and suppression is then sustained by the subsequent decomposition of more recalcitrant materials in the coarse and mid-sized particulate fraction (Stone et al. 2001).

Perhaps the most important feature of organic-matter mediated general suppression is its capacity to act against most, if not all, major soilborne pathogens of food and fibre crops. Since root disease problems in the field rarely involve a single pathogen, enhancing the suppressive potential of a soil with organic matter is one of the only non-chemical techniques available to control a suite of pathogens. This does not mean that manipulating organic matter to manage several pathogens is a simple matter. When pathogens which are good primary saprophytes but poor competitors are involved (e.g. *Pythium* and *Fusarium*), the fact that they may multiply on fresh organic matter before being suppressed must be taken into account when designing application strategies. In the case of *Rhizoctonia*, which has a high competitive saprophytic ability due to its capacity to degrade cellulose as well as simple sugars, organic-matter mediated general suppression is often insufficient to achieve control and specific antagonists may also be required (Stone et al. 2004).

1.4.2 Suppressing Nematodes with Organic Amendments

It has been known for many years that animal manures, oil-cakes, residues from leguminous crops and other materials with a low C/N ratio can be added to soil to control plant-parasitic nematodes (see reviews by Muller and Gooch 1982; Rodriguez-Kabana 1986; Stirling 1991). Although there is some evidence that such amendments increase populations of microorganisms antagonistic to nematodes, the main mechanism is thought to be the release of nematicidal compounds such as ammonia during the decomposition process. Since relatively high concentrations of ammonia are needed to achieve control, there is a direct relationship between the amount of N in an amendment and its effectiveness (Rodriguez-Kabana 1986). Thus amendments with N contents greater than 2% are usually used and application rates are typically greater than 10 t/ha.

Although the nematicidal effects of ammonia are well established (Eno et al. 1955; Rodriguez-Kabana et al. 1982; Oka and Pivonia 2002; Tenuta and Ferris 2004) and lethal concentrations are achievable with nitrogenous amendments, the

commercial use of such amendments is limited by cost and by concerns about the environmental impact of large quantities of nitrogen. Most recent studies have therefore sought to achieve efficacy at lower application rates. One successful approach involved adding a nitrification inhibitor (nitrpyrin) with the amendment to slow the oxidation of ammonia to nitrite and nitrate, therefore allowing ammonia concentrations to build up for an extended period. When the inhibitor was applied with chitin or cottonseed amendments, ammonia levels were higher for longer periods than in amended soils without the inhibitor, and this was associated with reduced egg production and galling from *Meloidogyne javanica* (Oka and Pivonia 2002). Alkaline additives have also improved the effectiveness of nitrogenous amendments by increasing soil pH and therefore shifting the equilibrium between the NH_4^+ and NH_3 to the latter form, which is nematicidal (Oka et al. 2006a).

Other work in the USA and Israel has shown that specially formulated organic amendments can cause nematode mortality through mechanisms other than ammonia production. De-watered municipal biosolids applied at 1.1% w/w did not affect *Heterodera glycines*, but the nematode was killed when the biosolids were stabilised with alkaline materials such as cement kiln dust, fly ash or quicklime (Zasada 2005). Nematode mortality was associated with a rapid increase in the pH of the soil solution (to a pH > 10), and this occurred when CaO in the amendment reacted with water to form $Ca(OH)_2$ (Zasada and Tenuta 2004; Zasada 2005). The contribution of ammonia production to the nematicidal effect was unclear in the American studies, but work with similar products in Israel suggested that it was important there (Oka et al. 2006b). However, the mechanism is clearly chemical rather than biological, as experiments with autoclaved materials indicated that microbes associated with the amendment were not involved (Zasada 2005).

Whether it will eventually be possible to use nitrogenous amendments in nematode management programs remains a moot point. Enormous quantities of organic and inorganic wastes and industrial by-products are available in most countries and there is a need to find uses for them as commercial fertilisers and soil conditioners. Alkaline-stabilised organic amendments are effective against plant-parasitic nematodes, but fine tuning will be needed before they can be used routinely in nematode management. Thus there is a need to determine the application rates required to achieve consistent nematode control; develop methodologies to prevent overproduction of ammonia and ensure that pH does not increase excessively; understand the long-term effects of these amendments on soil physical properties, soil chemistry and soil microbial ecology; and find ways of integrating the practice into the soil and crop management programs used for specific nematode-susceptible crops.

Although most recent research on organic amendments for nematode control has focused on nitrogenous materials, the possibility of using materials with a much higher C/N ratio has also received attention. McSorley and Gallaher (1995) used a composted mixture of sticks, leaves, branches, grass clippings and wood chips from the urban environment (C/N ratio=36) as an amendment or mulch and found that it had little effect on plant-parasitic nematodes in vegetable crops planted immediately after the amendment was applied. However, in another study that continued

for 3 years, population densities of plant-parasitic nematodes on maize were reduced in the third season, once the woody compost material had broken down and levels of soil organic matter had increased in amended plots (McSorley and Gallaher 1996).

Three studies in Australia have also shown that amendments with minimal amounts of N have suppressive effects on nematodes. In the first of these studies, apple trees mulched with sawdust for 5 years had much lower populations of *Pratylenchus jordanensis* in years 2–5 than non-mulched trees or trees growing in fumigated or nematicide-treated plots. In years 4 and 5, yields from mulched trees were as good as those obtained with methyl bromide fumigation (Stirling et al. 1995). A second study in which tomato was planted into field plots that had been amended over the previous 2 years with sawdust and urea showed that the amended soil was highly suppressive to *M. javanica* and that the level of nematode control was significantly better than that obtained with the nematicide fenamiphos. Plants in amended plots were almost free of galls, whereas the untreated controls were heavily galled (Vawdrey and Stirling 1997). The third study involved an amendment of sugarcane residue (the tops and leaves remaining in the field after sugarcane is mechanically harvested). Sugarcane was planted 23 weeks after the residue was incorporated into soil, and 24 weeks after planting there were 95% fewer lesion nematodes (*Pratylenchus zeae*) in roots growing in amended soil than in roots from the non-amended control (Stirling et al. 2005).

Results from these studies indicate that amendments with a high C/N ratio act much more slowly than nitrogenous amendments. When soil is amended with materials such as yard waste, sawdust or sugarcane residue, suppressiveness may take months or years to develop whereas it develops almost immediately when the amendment has a relatively high N content. Interestingly however, suppressiveness is soon lost with nitrogenous amendments. Thus when soil was assessed 4 and 7 months after it was amended with N-rich materials (e.g. lucerne hay, feedlot manure, poultry manure, chitin and a waste product from sugar mills known as mill mud), it was not suppressive to *M. javanica* or *P. zeae* (Stirling et al. 2003). In contrast, materials with a much higher C/N ratio (e.g. sawdust, sugarcane residue and grass hay) were suppressive to both nematodes.

Although the suppressiveness generated by high C/N amendments has not been studied in detail, the evidence currently available suggests that physical or biological rather than chemical mechanisms are responsible. Relatively large predators (e.g. nematodes and arthropods) may be able to operate more effectively when soil structure is improved with organic matter, while in the Australian studies discussed previously, one common observation was that fungi appeared to be associated in some way with suppressiveness. For example, a suppressive, sawdust-amended soil had high numbers of fungal-feeding nematodes (Vawdrey and Stirling 1997), while low concentrations of nitrate nitrogen, a fungal-dominant soil biology and high numbers of omnivorous nematodes were associated with suppression in one of the other experiments (Stirling et al. 2003). In an experiment where *P. zeae* was suppressed after soil was amended with sugarcane residue, an unidentified predatory fungus was found in the amended but not the non-amended soil (Stirling et al. 2005). It is therefore possible that fungal predation on nematodes was responsible

for these suppressive effects. The predatory hyphomycetes and several genera of wood-decaying basidiomycetes are commonly found in habitats that are rich in cellulose and lignin and are thought to have evolved the capacity to scavenge for additional N in low N environments by preying on nematodes (Barron 1992; Tzean and Liou 1993). Thus when high C/N amendments are added to soil, these fungi may utilise free-living nematodes as a food source and coincidently capture plant-parasitic species.

1.4.3   Farming Systems to Enhance General Suppressiveness

Although amending soil with high rates of organic matter can generate suppressiveness to nematodes and other soilborne pathogens and maintain it for some time after the amendment is applied, it is important to recognise that this approach to disease control is likely to be most useful in high value horticultural production systems. Nurseries where plants are grown in containers, glasshouses producing vegetable or ornamental crops and intensive in-field production of crops with a high monetary value are perhaps the only situations where it is realistic to use amendments in this way to manage nematodes. In all other agricultural systems, applying organic matter at rates of 10–100 t/ha/annum is never likely to be economically feasible. Importation of organic matter will generally be expensive relative to the income derived from most crops, largely because transportation costs are high and non-agricultural markets compete for the resource. Since high application rates are required to achieve the desired effects, there is also the potential for environmental problems from the nitrogen, heavy metals and other potential pollutants that may be present in the amendment. Thus for all the world's staple food and fibre crops, organic-matter mediated general suppression will mainly be achieved by developing farming systems that increase C inputs and conserve soil organic matter. Almost all soil and crop management practices affect the levels of soil organic matter, but perhaps the most important are crop rotation, cover cropping, crop residue management, organic amendments and tillage (Magdoff and Weil 2004). They are therefore the main tools that can be used to improve a soil's physical, chemical and biological status and therefore influence its capacity to suppress soilborne pests and pathogens.

Since accumulation of organic matter is directly related to C inputs (Paustian et al. 1997), reducing the frequency and duration of bare fallow periods and including perennial forages, high residue crops and cover crops within the farming system are the most practical ways of minimising the decline in soil organic C that occurs in all cropping systems. Careful management of above and below-ground plant residues also has a place, particularly in cropping systems where most of the above-ground material is harvested. Organic amendments may also be useful, but successive inputs at low application rates are likely to be more economically, agronomically and environmentally desirable than occasional inputs at high application rates. When used collectively, these practices are the first step towards increasing levels of soil organic matter and enhancing the suppressiveness of field soils to nematodes and soilborne diseases.

The second step involves reducing tillage, as conventional tillage arguably causes greater losses of soil organic matter than any other farm management practice (Magdoff and Weil 2004). In comparison to cultivated soils, non-tilled soils are less susceptible to erosion losses caused by water or wind, and decomposition also proceeds more slowly because crop residues remain on the soil surface rather than being mixed with the soil. Non-tilled soils are also cooler and subject to less pronounced wetting and drying cycles, both of which reduce rates of microbial respiration and organic matter decomposition. A compilation of studies from the literature (Franzluebbers 2004) indicates that soil under no tillage accumulates organic C to a greater extent than under inversion tillage, and that this effect is seen for both particulate organic matter and the more labile C fractions on which heterotrophic soil organisms depend. It is therefore not surprising that reducing tillage produces profound changes in the detritus food web, the most obvious impact being favourable effects on larger organisms such as predatory and omnivorous nematodes, mites, enchytraeids, earthworms, beetles and spiders (Wardle 1995).

When appropriate crop rotations, reduced tillage, residue retention, more frequent cover cropping and regular inputs of animal manures and organic wastes are integrated into a farming system, they are a powerful combination of practices that will result in improved soil and ecosystem health. Their widespread adoption in many industries in recent years is testimony to the benefits obtained. Although enhanced suppression of plant-parasitic nematodes will never be the primary reason for such improvements to a farming system, recent work on sugarcane in Australia suggests that it is one of the benefits that will accrue. Damage caused by *M. javanica* and *P. zeae*, the most important nematode pests of sugarcane, has been reduced by introducing a rotation crop and implementing residue retention and minimum tillage to enhance natural biological control mechanisms that suppress these pests (Stirling 2008). Although such suppressiveness is likely to take years to reach its full potential, particularly in farming systems where biomass production is limited by low rainfall, it is nevertheless worth pursuing because it comes with numerous other soil health benefits (e.g. improved nutrient cycling, better soil structure, increased water and nutrient holding capacity and broad-spectrum disease suppression) that are crucial for the long-term sustainability of a cropping system (Weil and Magdoff 2004). From the perspective of nematodes, future studies within improved farming systems should concentrate on establishing the levels of soil organic matter required to achieve suppression, understanding the regulatory mechanisms involved, and determining how the quality, quantity and timing of organic inputs influences the development of suppressiveness.

1.4.4 Specific Suppression of Soilborne Pathogens

There are many situations where soilborne diseases caused by fungi, bacteria or nematodes are suppressed by pathogen-specific agents. Historically, the best-documented examples for nematodes are the suppression of *Heterodera avenae* in

a cereal monoculture by two fungi, *Nematophthora gynophila* and *Pochonia chlamydosporia*, and the multiplication of *Pasteuria penetrans* in some cropping systems to levels that suppress root-knot nematodes. Both examples were discussed in detail by Stirling (1991).

In the last two decades, other examples of natural suppression due to *P. penetrans* have been reported (Weibelzahl-Fulton et al. 1996) and suppressiveness has been transferred from one field to another (Kariuki and Dickson 2007). The role of other *Pasteuria* species as suppressive agents has also been recognized, with Noel et al. (2010) demonstrating that when *P. nishizawae* is introduced into a non-suppressive field soil, it induces suppressiveness to soybean cyst nematode (*H. glycines*).

Another important contribution to our understanding of nematode-suppressive soils has been a decade-long investigation (reviewed by Borneman et al. 2004 and Borneman and Becker 2007) on the development of suppressiveness to *H. schachtii* in a field that had been cropped intensively with hosts of the nematode. After a period when populations of *H. schachtii* were high and disease incidence was severe, nematode populations declined to such an extent that studies commenced on the causes of the phenomenon. Work with various biocides (Westphal and Becker 1999) and experiments in which suppression was transferred to a conducive soil using either soil or cysts (Westphal and Becker 2000, 2001) showed that the suppressiveness was biological in nature and prompted studies of the microflora associated with nematode cysts and eggs. This work showed that eggs from the field were frequently parasitised by fungi and that *Brachyphoris* (syn. *Dactylella*) *oviparasitica*, *Fusarium oxysporum*, other *Fusarium* spp., *Paecilomyces lilacinus* and various unidentified fungi could be isolated on agar media (Westphal and Becker 2001).

The above investigations showed that fungi were associated with suppressiveness and subsequent studies demonstrated that modern technologies were useful for identifying the key suppressive organisms. Soils with different levels of suppressiveness were created with biocides or by combining different amounts of suppressive and conducive soil and oligonucleotide fingerprinting of rRNA genes (OFRG) was used to identify the main fungal phylotypes associated with different levels of suppression (Yin et al. 2003). The main phylotype in the most suppressive treatments had high sequence identity to rRNA genes from various nematode destroying fungi. Subsequent analyses indicated that the fungus represented by this phylotype was most closely related to *Brachyphoris oviparasitica*, a parasite of *Meloidogyne* eggs that had previously been found to suppress this nematode in California peach orchards (Stirling and Mankau 1978; Stirling et al. 1979). A second phase of the study validated this result, with sequence-selective quantitative PCR assays showing that the largest amounts of *B. oviparasitica* PCR product came from soils possessing the highest levels of suppressiveness to *H. schachtii* (Yin et al. 2003). In phase three of the study, *B. oviparasitica* was added to fumigated soil and produced the same high level and long-term suppressiveness that was observed in the naturally suppressive soil (Olatinwo et al. 2006a, b, c).

Other recent studies indicate that when field soils are surveyed systematically for suppression using appropriate techniques, examples of specific suppressiveness