13 Biological and Cultural Management

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13.1. Introduction

Biological control of nematodes principally concerns the exploitation of microbial agents. As cultural methods of crop protection, including cultivation and applications of organic matter (soil amendments), have profound effects on microbial abundance, diversity and activity in soil, it is appropriate that both approaches to nematode management are considered together. Several aspects of biological and cultural control have been reviewed (Stirling, 2011; Timper, 2011).

This chapter is directed towards the **management** of nematodes as opposed to their **control**. Control implies the use of a single measure to reduce or eliminate nematode pests, which in most cases is not possible, whilst management involves the manipulation of nematode densities to non-injurious or sub-economic threshold levels using several measures with consideration of the whole production system; maintenance of diversity is an objective of management but not of control (Brown and Kerry, 1987). Consequently, of increasing importance is the additional need to take into consideration the impact of the pest management strategies on biodiversity and the ecological balance in the soil.

Biological control is defined here as the management of plant diseases and pests with the aid of living organisms. This definition includes predators and parasites of organisms that kill or damage their hosts and also microbes that indirectly influence the establishment, function and survival of pathogens and pests. The plant may have significant influences on these interactions but plant resistance is not considered as a component of biological control. Neither is plant resistance discussed here as a cultural management tactic because it is the main topic of Chapter 14. It should be noted, however, that resistant rootstocks can influence the soil microbial populations around roots. Grafting of preferred, but nematode susceptible, cultivars onto hardier, nematode-resistant rootstocks has long been an accepted practice for perennial crops, such as coffee, and well as for vegetables like tomato, and is gaining more popularity. More direct changes in the plant rhizosphere through the incorporation of organic materials, which influence microbial communities and help manage diseases and pests, are considered as **cultural control** in this chapter. The link between biological control and cultural methods becomes evident when the exploitation of natural enemies as biological control agents for the management of plant-parasitic nematodes is discussed. Both biological and cultural control methods are important for the biomanagement of pests through the combination of measures other than chemical pesticides to improve soil quality and plant health. The application of bioactive compounds, which are derivatives of plants or metabolites derived from fungi or bacteria used as bio-nematicides, are discussed (see Chapter 16) but are not considered as biological control. Although many organisms derive their nutrition from nematodes, the most studied natural enemies of nematodes are bacteria and fungi.

There is considerable public and legislative pressure to reduce the use of nematicides because of their potential health and environmental risks but few organisms have been developed as practical control agents and none is in widespread use. Apart from microbially-induced suppression of nematode pests, research suggests that biological control agents will generally provide too little control to be effective alone and their successful use in sustainable management strategies will depend on their integration with other control measures. These include the use of genetic resistance, quarantine measures and various cultural control practices. Numerous reviews on the use of cultural control practices for nematode management are available (e.g. Chen et al., 2004; Luc *et al.*, 2005; Perry *et al.*, 2009). A single management option rarely leads to the sustainable management of a nematode problem. A successful nematode management strategy ideally will involve the selection of a combination of complementary components, providing they are applicable, appropriate and economical. Furthermore, a successful strategy should be flexible to pest and disease changes, as cropping systems and knowledge evolve over time. Selection of a practice inevitably depends on a multitude of considerations, not least the scale of the cropping system; a smallholder farmer in Africa, for example, has wholly different criteria against which to assess a situation than does the large-scale intensive cereal farmer in North America or the highly intensive ornamental crop producer in Europe. Thus, biological and cultural nematode management options described here are not necessarily universally applicable and must be adapted to meet local needs. Such considerations are especially true for exploiting cultural control methods, such as the use of bulky soil amendments that may be only locally available. Moreover, much of the information on biological and cultural control practices emphasises the importance of an understanding of the target plant-parasitic nematode including its identification, hosts and environmental preferences. However, small-scale farmers, especially those in developing countries, who would benefit much from these management strategies, have little knowledge or access to such information. Therefore, they need technical guidance and assistance from well-informed extension people with adequate training in nematology, which, in many circumstances, is also sorely lacking.

13.2. Suppressive soils

Soils are considered suppressive when nematode multiplication on susceptible crops is less than that normally observed on the same cultivar in another soil, in similar abiotic conditions. Many soils that are suppressive to nematodes are often not recognized as such, because nematode problems have not occurred for several years. However, observations on abnormal declines in nematode populations or their damage in fields where plant-parasitic nematodes used to be a problem have led to the discovery of natural biological control and the organisms involved. Methods have been developed to assess suppressive soils (Box 13.1).

Most organisms found to be involved in nematode suppression are nematophagous fungi (e.g. *Pochonia chlamydosporia* (synonym *Verticillium chlamydosporium*)*, Hirsutella rhossiliensis, Dactylella oviparasitica* and *Trichoderma* spp.) and bacteria (e.g. *Pasteuria penetrans*) that parasitize their nematode hosts. Microbes that compete for nutrients, produce toxins or induce host resistance, such as some rhizosphere bacteria (e.g. *Pseudomonas* spp., *Agrobacterium radiobacter*, *Bacillus subtilis*) may reduce nematode damage but may not provide the long-term control of nematode populations associated with suppressive soils.

Box 1.1. Methods to assess the extent and nature of suppressive soils**.**

- Comparison of nematode multiplication in untreated soil and soil treated with a chemical or physical biocidal treatment.
- Inducing suppression by the transfer of microbial agents from a suppressive to a conducive soil.
- Addition of different numbers of infective nematodes to a conducive and a suppressive soil and compare nematode reproduction.
- Correlation of population densities of specific organisms in soils with increased levels of suppressiveness using biological, culturing and molecular methods to measure their abundance and activity (Borneman *et al.*, 2004)

Studies of suppressive soils have led to the identification of several important potential biological control agents, which may be abundant and occur in many fields and orchards, and even in natural environments (Stirling, 2011). Natural biological control may be widespread but, in most cases, it is inadequate to keep nematode populations below their damage threshold densities for crops grown in most agricultural systems. The challenge is to determine the specific conditions that are conducive for biological control agents to work in agricultural systems so that we can manipulate the environment, or the biological control agents, to suppress population densities of plant-parasitic nematodes adequately.

13.3. Biological control agents

It is not possible to include here all the natural enemies of nematodes that have been described and studied. Instead, we give an overview of the major groups with potential as biological control agents and include details of some of the most studied organisms.

13.3.1. Predators

Predatory nematodes, mites, insects and several other invertebrates, such as tardigrades, feed on nematodes. Predators are common in soil and can also feed on other organisms. The biology and feeding habits of some predatory nematodes, e.g. *Mononchoides gaugleri*, have been studied in detail. They can destroy several nematodes per day using their teeth, enzymes or toxins to kill their prey. Micro-arthropods, such as mites and springtails, have a role in the regulation of nematode populations, especially in natural ecosystems where they may be abundant. However, their lack of specificity for plant-parasitic nematodes makes them unsuitable for use in biological control programmes aimed at specific nematode pests. Also, their mass production and delivery to the soil is considered impractical.

13.3.2. Nematophagous fungi

Some nematophagous fungi are obligate parasites (Box 13.2), which need nematodes to survive, others are facultative or opportunistic parasites (Box 13.3), which can survive saprophytically, and others have characteristics that are intermediate between these two categories. Nematophagous fungi may be most readily divided into those that have extensive hyphal growth outside their hosts, such as the nematode-trapping fungi, and the opportunistic parasites of nematode eggs and those fungi that are mainly endoparasitic. The trapping fungi immobilize nematodes using non-adhesive traps or sticky structures usually produced on mycelia before they infect their host. Opportunistic fungi colonize the rhizosphere and attack the sedentary stages (females and eggs) of nematodes developing on plant roots. The endoparasitic fungi penetrate nematodes after germination of their adhesive spores, which attach to the nematode cuticle. Nematode-trapping fungi ensnare active nematodes using one or more types of mycelial trap. For example, *Arthrobotrys dactyloides* uses constricting rings, *Dactylella candida* makes non-constricting rings and adhesive knobs, *Monacrosporium cionopagum* forms adhesive branches and a two-dimensional adhesive network, whilst *M. ellipsosporum* traps nematodes with adhesive knobs or adhesive branches that may form loops. Some nematode-trapping fungi are good saprophytic competitors but trap few nematodes, while others are efficient in capturing nematodes but do not establish well in soil. Little is known about their growth and development in soil, especially the factors which cause the switch from the saprophytic to the parasitic phase. The trapping fungi apparently need a carbohydrate source to proliferate but other factors, such as those leading to mycostasis (inhibition of spore germination and/or growth caused by the residual microflora), also play a role in their abundance and trophic state in soil. Recent research exploiting genomics has begun to investigate the key molecular mechanisms involved in the interaction between trapping fungi and nematodes (Tunlid and Ahrén, 2011). Difficulties in the establishment of these fungi in the soil, their limited trapping activity and especially their non-specific capture of plant-parasitic nematodes reduce their potential in biological control. Some *Arthrobotrys* spp. have been formulated and applied under specific conditions, but with inconsistent results.

Box 13.2. Obligate parasites.

Types of Organism: *Pasteuria penetrans*

Mode of Action: Parasitism via adhesive spores.

Advantages:

- virulent against active and sedentary nematodes
- resting spores with long shelf life
- responsive to pest densities

Limitations:

- difficult to produce *in vitro*
- limited spread in soil
- narrow host range

Arthrobotrys oligospora is the best known and most studied nematode-trapping fungus. It makes a three-dimensional hyphal network to trap soil-dwelling nematodes. The network is coated with an adhesive that contains lectins that bind to specific carbohydrates present on nematode cuticles. The network attracts nematodes more than vegetative mycelium and the presence of nematodes induces trap formation. However, most studies were performed *in vitro* and it is not clear if these interactions are relevant in soil. Immobilization of the nematodes may be enhanced by a nematotoxin produced by the trap. Once the nematode is trapped, a hypha penetrates the cuticle within 1 h and forms an infection bulb. Penetration is thought to be mostly mechanical but collagenase might play a role. Assimilative hyphae develop and the fungus colonizes the nematode body. Later, conidiophores develop from the cadaver and bear conidia in a succession of clusters.

Arthrobotrys oligospora is a good saprophytic competitor capable of using a wide range of carbohydrates. As with most trapping fungi, it captures all kinds of nematodes. The fungus is also able to colonize plant roots and cause cell wall modifications without affecting plant growth.

Box 13.3. Facultative parasites.

Types of Organism**:** Trapping fungi; parasites of nematode eggs.

Mode of Action**:** Parasitism via traps produced on modified mycelium and/or penetrative hyphae.

Advantages:

- easily produced *in vitro*
- some species rhizosphere competent
- wide host range

Limitations**:**

- may be difficult to regulate switch from saprophytic to parasitic activity
- efficacy dependent on plant species, nematode host and other soil conditions that effect saprophytic growth
- several species do not form resting structures and may be difficult to formulate

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Many fungi infecting vermiform plant-parasitic nematodes form adhesive spores that adhere to the cuticle of the nematode when it passes the fungus. These fungi are mostly obligate parasites and poor saprophytic competitors in soil but generally have a broad nematode host range. For example, *H. rhossiliensis* (Fig. 13.1) was found to reduce nematode invasion, and consequently nematode populations, of *Meloidogyne javanica*, *M. hapla*, *Heterodera glycines* and *Criconema xenoplax*. Other examples of similar endoparasitic fungi that have been studied more closely are *Drechmeria coniospora, Nematoctonus* spp. and *Verticillium balanoides.* Not all endoparasitic fungi produce adhesive spores; some form zoospores that swim to the nematode cuticle, attach and encyst, often around the natural openings of the host. These encysted zoospores form a penetration tube that enters the nematode body, which the fungus colonizes by hyphal growth. The hyphae differentiate into sporangia where zoospores are produced. Some zoosporic fungi, such as *Catenaria anguillulae*, which attack vermiform nematodes, are believed to be opportunistic but others, such as *Nematophthora gynophila* and *Catenaria auxiliaris*, infect sedentary young female cyst nematodes and are obligate parasites. Zoospores require water-filled pores to be active and nematode infection is much affected by soil moisture levels. As many parasites of vermiform nematodes and the zoosporic fungi are difficult to culture *in vitro* and establish in soil, they are considered unsuitable for large-scale application as biological control agents.

By contrast, facultative fungal parasites of sedentary stages (eggs, developing juveniles and females) have attracted most interest because of their potential as biological control agents. Their target pest is immobile, and thus easier to infect, and these facultative parasites are able to survive saprophytically in the rhizosphere and most are relatively easy to mass-culture. They are not as specialized as the fungal parasites attacking soil-dwelling nematode stages and usually infect their host by simple hyphal penetration, sometimes with the formation of an appressorium. Many kinds of fungi have been isolated from sedentary stages but few have been studied in detail. *Cylindrocarpon destructans*, for example, is found regularly, as are species of *Fusarium*, *Gliocladium*, *Pochonia* and *Trichoderma,* all opportunistic fungi whose pathogenicity to nematodes may differ considerably between isolates. *Purpureocillium* (synonym *Paecilomyces*) *lilacinus* is a well-studied parasite of a number of nematodes, including *Radopholus similis* and *Tylenchulus semipenetrans*, but most research has focused on the parasitism of *Meloidogyne* spp. and *Globodera rostochiensis* eggs. The fungus is abundant and active in sub-tropical and tropical regions. It is effective in reducing nematode damage to a range of crops in field trials and has been widely evaluated and developed by several small companies around the world. Soil application of the fungus has often resulted in variable levels of nematode control, although improvements through commercial development indicate that more consistent and promising results may be obtained. It is marketed in Germany (BioAct®WP) and in South Africa (Pl Plus®) for cyst and root-knot nematode management. Both products use the same strain 251 of the fungus, which is applied as dispersible granules for application in water.

Pochonia chlamydosporia parasitises females and eggs of cyst and root-knot nematodes (Fig. 13.2). Hyphae penetrate eggs after formation of an appressorium on the eggshell. A serine protease and chitinases, which degrade the eggshell, and a nematotoxin, phomalactone, produced by *P. chlamydosporia* may be involved in pathogenicity. Chlamydospores, which are resilient to environmental extremes, are produced as a survival structure and are used as an inoculum to establish the fungus in the soil and rhizosphere. Isolates of the fungus differ greatly in their ability to produce chlamydospores, colonize roots and infect nematodes. A range of biological (e.g. dilution plating on a selective medium) and molecular (polymerase chain reaction (PCR), real-time PCR, restriction fragment length polymorphism (RFLP)) methods have been developed to monitor the occurrence, abundance and activity of the fungus in the soil, rhizosphere and nematode egg masses. Rhizosphere colonization differs with plant species and is improved when the plant is infected by nematodes. The fungus also has host preferences: isolates obtained from root-knot nematodes are less able to infect cyst nematode eggs than isolates originating from cyst nematodes

(Kerry and Hirsch, 2011). Single applications of the fungus at rates of 5000 chlamydospores g^{-1} soil have provided control of root-knot nematodes on vegetable crops in tropical soils but results in Europe have been less satisfactory. The fungus is more effective when applied to low, pre-cropping densities of root-knot nematodes, preferably on poor hosts for the nematode, than when applied to large nematode infestations on highly susceptible crops. The factors that control the switch from saprophytic to parasitic activity are poorly understood. Even though *P. chlamydosporia* may be more abundant in soil with large amounts of organic matter, its parasitic activity may be no greater than in a mineral soil. Formulations based on fungal hyphae and conidia have been developed but chlamydospores are the most robust form of inoculum. Toxicological tests on chlamydospore-based inoculum have been successfully completed with products expected for more widespread evaluation.

Trichoderma spp. commonly occur in soil and have received increasing attention for nematode management; isolates have been identified that successfully antagonise and control a wide range of plant pathogens including nematodes (Sharon *et al*., 2011). Recent research, focused on strains *T. asperellum*-203 and *T. atroviride* IMI 206040, previously defined as strains of *T. harzianum*, has shown control activity against *M. javanica*. Variability in the efficacy of different strains has been shown and the application of mixed strains has led to increased levels of nematode control with some strain combinations but not others. This result indicates differing modes of action and, indeed, studies have focused on conidial attachment and parasitism, revealing that a wide range of lytic enzymes (proteases and chitinases) are produced, the induction of which is likely to be environmentally mediated (Sharon *et al*., 2011). When searching for strains suitable for the management of root-knot nematodes, studies indicated that isolates from egg masses are more effective than those from soil. Furthermore, assessing the genetic potential to produce chitinolytic enzymes was suggested as an additional selection criterion.

13.3.3. Endophytic fungi

Endophytic fungi (Box 13.4) grow within plant tissues without causing disease. Arbuscular mycorrhizal (AM) fungi, e.g. *Glomus* spp., are the best known endophytes associated with plant roots; they are obligate symbiotic parasites of plants. Their role in protecting the plant from nematode damage and in reducing nematode densities in the soil has been studied in different plant-nematode interactions, but most involve *Meloidogyne* spp. (Fig. 13.3) with only few studies looking at cyst and migratory nematodes (Hallmann and Sikora, 2011). AM fungi enhance plant growth by improving plant access to nutrients, especially P, and particularly under conditions of poor nutrient availability. AM fungi also aid access to and uptake of water, alleviate heavy metal toxicity and suppress pest and disease damage including that of nematodes. Colonisation of roots by AM fungi before nematode invasion may reduce nematode multiplication rates to a greater extent than after nematode invasion. The specific mode(s) of action of nematode antagonism is not well understood but are thought to range from very specific mechanisms to several mechanisms working in concert. They may also interfere with the production of root diffusates or produce nematotoxic compounds. AM fungi are produced commercially as crop growth enhancers. Other endophytes, such as *Neotyphodium* spp. in the leaves of grasses and asymptomatic and non-virulent strains of *Fusarium oxysporum* in banana and tomato roots, may rely on a toxic mechanism to reduce nematode infestations in roots. Increasing interest in endophytic pest and disease management has broadened the scope of research activity, with various fungal and bacterial isolates being assessed for potential use. The levels of nematode management can be acceptable, although isolates of the same species differ markedly in their efficacy against nematodes. Few, if any, commercial products based purely on endophytic activity are currently available, although isolates of *F. oxysporum* active against *Radopholus similis* have undergone development and field testing on banana plantations in Central America and East Africa. Again the modes of action are poorly understood, but induced resistance plays an important part in the interaction.

Box 13.4. Endophytes.

Types of Organism:	Arbuscular mycorrhizal (AM) fungi and other non-pathogenic root-colonising fungi and bacteria
Mode of Action:	competition for space and nutrients in roots and/or antagonism, increased (systemic) host defence/tolerance

Advantages:

- active against wide range of nematodes including migratory endoparasites in roots
- may suppress nematode colonization and multiplication
- may promote plant growth and reduce nematode damage
- easily produced *in vitro* and formulated
- may be applied as a seed/seedling treatment

Limitations:

- non-mycorrhizal fungi are closely related to plant pathogens and may be difficult to register for release
- activity affected by crop cultivars

13.3.4. Bacteria

Most bacteria that interfere with nematode behaviour, feeding or reproduction do so indirectly by producing antibiotics, enzymes or toxins (Box 13.5). Many products, such as volatile fatty acids and nitrogenous substances, are formed by bacteria during decomposition of organic materials and may influence nematode populations in the soil and rhizosphere. Screening rhizobacteria or their metabolites (extracts of their cultures) in Petri dishes has led to the discovery of bacterial strains with strong antagonistic properties. However, the production and importance of such metabolites in the rhizosphere is not clear. *Burkholderia* spp., *Pseudomonas* spp., *Bacillus* spp. and *A. radiobacter* may reduce nematode invasion of roots through effects on nematode hatching and mobility or may induce plant resistance. Several of these bacteria are also plant-growth promoting bacteria (PGPB).

Box 13.5. Antagonists.

Types of Organism:	Rhizosphere bacteria.
Mode of Action:	Toxins; modification of root exudates and/or induced resistance.

Advantages:

- easily produced *in vitro*
- may be applied as a seed/seedling treatment
- active against a wide range of plant-parasitic nematodes
- may promote plant growth and reduce nematode damage

Limitations:

- may be effective for a relatively short time
- no direct effect on nematode multiplication
- activity affected by crop cultivar and soil environment

Of the few bacteria known to parasitize nematodes, *Pasteuria* spp. are endospore-forming bacteria and show greatest potential to be developed into biological control agents. *Pasteuria* endospores have been observed adhering to and parasitizing all of the economically important plant-parasitic nematodes. *Pasteuria* species and isolates show varying degrees of host-specificity; for example, a *Pasteuria* population isolated from cyst nematodes appeared to be more promiscuous than those isolated from root-knot nematodes (Mohan *et al*., 2012). Molecular studies have led to the establishment of phylogenetic relationships between *Pasteuria* spp. and have provided tools to test if plants are infected with *Pasteuria*-infected nematodes (Rao *et al*., 2012). The life cycle of *Pasteuria* spp. is initiated when dome-shaped endospores adhere to the cuticle as the nematodes move through soil (Fig. 13.4). It is thought that collagen-like fibres on the surface of the endospore are involved in a Velcro-like attachment process with a cuticle receptor (Davies and Curtis, 2011). Depending on the nematode species, endospores germinate either immediately (e.g. in *Heterodera avenae*), or later when the nematode has entered the root and initiated a feeding site (e.g. *Meloidogyne* spp.) by producing an infection peg that penetrates the cuticle. Following germination, small rod-shaped bacteria develop exponentially to produce granular masses that eventually enter sporogenesis and form the next generation of spores (Davies *et al*., 2011). In *Meloidogyne* spp. the infected female continues to develop, becoming infertile as up to 2 million endospores are produced by the bacterium destroying the reproductive system. These endospores are released into the soil upon decomposition of roots and infected females, providing new inoculum of the biocontrol agent. Spores can survive in air-dried soil for several years, but successful infection of nematodes greatly depends on the distribution of the spores in the soil, which can be influenced by soil type, tillage practices, moisture and temperature. Juvenile nematodes in subsequent generations on the same plant only move short distances from the egg to nearby roots and are less infective than those in the first generation.

A natural decline of root-knot nematodes in fields of tobacco was shown to be caused by *Pasteuria* spp. but most studies on the efficacy of the bacteria as a biological control agent have been performed in pots because of difficulties in producing sufficient spores for large-scale trials. Declines in nematode numbers and root galling have been reported in small plots. Application is complicated because some isolates of the bacterium are very host specific and spore burden is not always correlated with virulence. The need for thorough distribution of spores around the roots requires inundative release of the bacteria at rates of $> 10^4$ spores g^{-1} soil. A main disadvantage of *Pasteuria* spp. for use in biological control was its obligate parasitism and hence difficulty for mass production, ~~but breakthroughs at Pasteuria Bioscience Inc. (USA) have led to *in vitro* culture of *Pasteuria* spp. Already *Pasteuria* spp. parasitic on sting nematode (*Belonolaimus longicaudatus*) is being produced *in vitro* and marketed as Econem® for nematode control on golf greens. The availability of mass production for niche markets paves the way for field scale applications. Once more is known about the specificity of the populations towards different nematode species, *Pasteuria* spp. have increasing potential to be a most successful biological control agent.~~
but breakthroughs at Pasteuria Bioscience Inc. (USA) in the first decade of this century led to developments in the *in vitro* mass production of *Pasteuria* that have been taken up by Syngenta Seedcare and used in their seed dressing technologies in which *Pasteuria nishizawae* was incorporated into a product called *Clariva*™ and marketed for the control of the soybean cyst nematode *Heterodera glycines*.

13.4. Interaction with Rhizosphere Microflora

The soil microflora in the rhizosphere is influenced not only by soil treatments, such as application of organic matter or soil disinfestation, but also by the presence of nematodes and by the plant species and cultivar. The population dynamics of the rhizosphere microflora are the result of direct and indirect effects of multitrophic interactions between the plant roots, the plant-parasitic nematodes and their natural enemies. These complex interactions between micro-organisms and their environment are poorly understood. For example, plant root exudates of nematode host plants were found to alter second-stage juvenile cuticle aging, and thereby making the nematodes increasingly susceptible to hyperparasitic bacteria, whereas root exudates from non-host did not (Mohan et al., 2020). These types of subtle interactions are only just being discovered in relationship to plant-parasitic nematodes, but in entomological situations such interactions have been developed into sustainable pest control strategies (Cook *et al*., 2006). These multitrophic interactions between rhizosphere micro-organisms,microbial biological control agents and plants are complex and may also involve competition for space and nutrients as well as antibiosis.

Molecular techniques, and more recently the use of metagenomic approaches, offer great possibilities to study changes in the microbial community of the root microbiome as species and isolates of micro-organisms can be more easily monitored than with classic tools such as selective media. For example, the bacterial microbiomes have been investigated in nematode conducive and suppressive soils (Elhady *et al*., 2017) and it is expected that with these new techniques, increasing insight will be gained on the role of soil biodiversity and in the mechanisms of ecosystems resilience, i.e. the capacity of an ecosystem to respond to disturbance and recover quickly. This knowledge could possibly be used to improve mechanisms for introduction or rather enhancement of biological control agents in agricultural soils where plant-parasitic nematodes cause unacceptable yield reductions.

13.5. Application of Biological Control Agents

Successful biological control much depends on a thorough understanding of the population dynamics of the natural enemies and target pest, and of their interactions. For many organisms that have potential as biological control agents for nematodes such information is lacking.The pioneering work of Jaffee *et al.* (1992) in model systems revealed that parasitism by some microbial biological control agents is density-dependent, i.e. the probability of a specific host being parasitized increases with the density of the host. Although the activity of facultative parasites, including some nematode-trapping fungi, is density-dependent, their ability to survive saprophytically and their wide host range should enable them to be more effective at low pest densities than obligate parasites, such as *P. penetrans*, or those with poor saprophytic survival capabilities, such as *H. rhossiliensis*. The size of the inoculum reservoir required for significant infection rates and the relatively low transmission rates in soil tend to dampen fluctuations in the dynamics of the interactions between nematodes and their microbial parasites and pathogens. It is now becoming increasingly acknowledged that plant roots are adept at subtlety manipulating their microbial communities to their own advantage (Cox et al., 2019) and therefore a thorough understanding of these multitrophic interactions, at both the molecular and population level, needs to be placed within a theoretical framework if the development of rational biological control strategies is to be successful.

Currently, the application of biological control agents for the management of plant-parasitic nematodes in the field is notoriously difficult and most empirical studies have provided inconsistent results. However, some agents may be practically exploited in nematode management and some, such as *Purpureocillium lilacinus*, *Trichoderma* spp. and *Pasteuria* spp. *Pochonia chlamydosporia*, have been commercialized. The steps towards commercialization include selection of the most effective isolates, production of inoculum and formulation of the microbial agents. As nematicides continue to be prohibited due to their toxic effects it is important to understand that biological control cannot be a simple replacement for chemical control; application of microbiological control agents should not be considered as a silver bullet, but needs to be viewed within a framework of other management strategies, especially their interaction with cultural control methods (Stirling, 2011; Timper, 2011).

13.5.1. Selection of biological control agents and isolates

Due to the complexity of interactions that take place in a biodiverse rhizosphere, the choice of a biological control agent will depend on the nematode species and nematode stage to be controlled, as it is clear that some organisms are host-specific or attack only certain developmental stages and others feed on many kinds of nematodes. A combination of biological control agents with different modes of action or range of environmental tolerance limits may result in improved nematode control. However, no benefits and even reduced effects can occur and so careful selection of organisms for combination is required to avoid competition or antagonistic effects (Meyer and Roberts, 2002). In practice, combinations of agents may be too expensive to produce and register and other approaches such as soil amendments (see 13.6.3) may be considered.

Comparisons of different isolates of nematophagous fungi and bacteria have revealed that there exist enormous intraspecific differences in the performance of the microorganisms as potential biological control agents. Performance refers to the ability to parasitize the nematode and also to the survival capacity in soil under different environmental conditions (e.g. the production of survival structures, saprophytic growth), establishment in the rhizosphere (root-colonising capacity) and soil (dependence on nematode hosts and production of resting structures). With improved knowledge on their biology, it is possible to focus selection for biological control agents. For example, when assessing *Trichoderma* spp.

as biological control agents of *Meloidogyne* spp., those isolated from egg masses of *Meloidogyne* spp. appeared more effective than isolates recovered from soil (Affokpon *et al.*, 2011). In general, screening programmes to select isolates with biological control potential have resulted in < 10% of the isolates being selected in simple bioassays and more being rejected in tests in soil. Hence, focus on selection of appropriate isolates is a key stage in the development of a biological control agent. However, care should be taken when interpreting results. For example, a recent study revealed that the egg parasite *Pochonia chlamydosporia* was responsible for the suppression of *Meloidogyne* spp. in vegetable production systems in Spain (Ghahremani *et al*, 2022); their interpretation was similar to earlier studies of Kerry *et al*., (1982) where a fungal egg parasite was held responsible for the suppression of *Heterodera avenae* in cereals. However, it was later revealed that in the same field a *Pasteuria* population was infecting over 50% of the migratory infective juveniles and prohibiting them from reaching the root (Davies *et al*., 1990) suggesting a mixture of microbes was responsible for the suppression of cereal cyst nematodes overall but each acting on different stages of the nematode's development. Therefore, this demonstrates there was a diversity of species and isolates of *P. penetrans* and *P. chlamydosporia* within a single site; such diversity may enable the community to function in different environmental conditions, on different parts of the root system and against a variable host; it is unlikely a single isolate of a specific agent can be used as a single management strategy.

13.5.2. Inoculum production and formulation

Most fungi and bacteria with activity against nematodes are not obligate parasites and can be cultured on artificial media or grain products (e.g. wheat bran). Mass production of microbes on artificial media can undergo a process of attenuation whereby the microbe loses its ability to parasitize its host; it is therefore important that the efficacy of the biological control agent is maintained, even after frequent sub-culturing. Although regular passage of the isolate through the nematode host to maintain its pathogenic capability has been recommended (Crump, 2004), it is not clear if this is important for facultative parasites that establish in soil through saprophytic growth.

Once inoculum has been produced, it needs to be formulated for storage. An ideal formulation has a long shelf life, allows for easy transportation and application, is readily distributed in soil and provides optimum conditions for growth and survival of the introduced agent. The formulation must also be compatible with other crop protection measures. For example, there is a whole science around the development of seed coatings whereby each individual seed is encased with a small amount of fertiliser together with a number of fungicides, pesticides and biologically beneficial microbes that enhance growth of the germinated seed and protected it from an array of soil borne pests and diseases (Rocha *et al.*, 2019). It is therefore imperative that the different components of the seed coating are mutually compatible to each other and not antagonistic. Alginate pellets have often been used to formulatespores and mycelial fragments of several fungi have been tested and were shown to be an efficient way to store, distribute and apply the fungi while maintaining biological control activity. However, the pellets and their contents may be destroyed by collembola and mites and at high concentrations alginates may be phytotoxic. Together with the biological control agents, amendments, such as chitin, can be added as a food source for the microflora. However, this practice does not always result in increased parasitic activity of the biological control agent.

I have deleted here the whole section from "Together with the biological control agents...

… decreased concomitantly with numbers of their nematode host."

DANNY PERHAPS YOU COULD DO SOMETHING HERE ABOUT BANANA PAPER AND ABERMECTINS?????????

13.6. Integration of Biological Control with Other Control Measures

The development of biological control strategies may involve the application of a biological control agent, but also the use of methods to increase the impact of the residual soil microflora on pest nematode population dynamics.

13.6.1 Crop rotation

As biological control agents alone rarely provide adequate control, they should be integrated with other methods such as crop rotation, resistant cultivars or antagonistic plants either to reduce the nematode populations in soil or to promote the establishment of the biological control agent (Kerry and Hominick, 2002). Different crops produce different root diffusates and these affect microbial activity in the soil and rhizosphere but little is known of their effects on the performance of biological control agents. The rhizosphere-colonizing capacity of *P. chlamydosporia* differs greatly among host plants and nematode control is greatest on roots that support extensive fungal growth but are poor hosts of the nematode. Hence, most nematode control is achieved by combining the fungus with a poor host for the plant-parasitic nematode to reduce the infestation in soil before the next susceptible host.

13.6.2. Soil disinfestation

Several control measures such as soil disinfestation through soil solarization, biofumigation, steaming (see 13.9) or application of broad-spectrum biocides (see Chapter 16), facilitate the establishment of biological control agents introduced after these treatments. As the activity of the soil microbial community is reduced, the establishment and survival of biological control agents is enhanced, at least in the short term. Soil disinfestation will also reduce the densities of plant-parasitic nematodes to levels that are manageable for biological control agents.

13.6.3. Soil amendments, green manures and biostimulants

In contrast to soil disinfestation, addition of organic matter to the soil through soil amendments, green manures and biostimulants results in an increase in numbers and diversity of microbial populations, including beneficial ones. Although there is a certain amount on controversy as to what is and what is not a biostimulant (Yakhim *et* al., 2016) all these approaches generally rely on the application of organic matter, without the external addition of a specific biological control agent (D'Addabbo *et al.,* 2019) , and this often results in suppression of plant-parasitic nematode populations (see section 13.11.1) and increase in saprophytic nematodes. The mechanism of this nematode suppression can be attributed to the release of nematicidal compounds by the organic material (e.g. glucosinolates) (Fleming *et al*., 2006), to the production of allelochemicals (e.g. antibiotics, chitinases), by the soil microflora that was able to increase in numbers because of the amendments, and to improved plant growth (e.g. by plant-growth promoting bacteria) (Widmer *et al.*, 2002; Thoden *et al.*, 2011). It is assumed that the incorporation of organic matter increases the abundance of microorganisms active against nematodes. However, addition of organic amendments may increase microbial competition and result in reduced nematode parasitism by poor competitors such as *H. rhossiliensis*. Addition of organic amendments may affect the switch from the saprophytic to the parasitic state and some facultative parasites are less active in soils following application of soil amendments. The presence of adequate nutrients, supplied by the organic amendments, may suppress the production of enzymes that aid in nematode infection yet provide abundant materials and energy for population increase of the biological control agent. For example, the serine proteases from *P. chlamydosporia, P. lilacinus* and *P. suchlasporium* are repressed by the presence of glucose and easily metabolized nitrogen sources.

Defined amendments have also been used for nematode management. The addition of chitin causes an increase in chitinase-producing microbes in the soil and rhizosphere, which are thought to degrade the chitin-rich eggshells of nematodes. Although there may be an increase in the abundance of nematode antagonists after such applications, the major effect of chitin on nematodes, especially soon after application, is due to the release of ammonia. In general, large amounts of soil amendments are needed to reduce nematode infestations significantly and their effects on the soil microbial community are complex and difficult to interpret. There is also interest in the use of chemical elements (e.g. silicon) that increases the general resilience of the plant to crop pests and diseases; for example, priming rice roots with silicon induced a defence response that protected plants against the root-knot nematode *Meloidogyne graminicola* (Zhan *et al*., 2018). With the growing need for alternatives to chemical nematicides these approaches are going to increase.

13.7. Nematode-free Planting Material

Problem avoidance should essentially form the basis of any pest management strategy. Use of nematode-free planting material provides an effective means of nematode management (or avoidance), often at a fraction of the cost fiscally and environmentally, to treating the cropping area, which may be impractical in any case. This nematode management tool requires that farmers understand the benefits of more costly, healthy planting material.

13.7.1. Production

Healthy planting material may be available through the use of certified seed and planting material, the production of seedlings/plantlets in sterile conditions and/or an effective quarantine system (see Chapter 12), which prevents the introduction of foreign species. Cropping systems using nursery-grown plants for transplantation afford an excellent opportunity to provide nematode (and other soil-borne disease) protection. Such strategies will limit nematode transmission to otherwise pest-free areas. In developing countries, subsistence farmers could prevent major losses from nematodes by using simple nurseries for nematode-free seedling production. Inert or sterilised potting media (e.g. sawdust, coconut husk, peat, vermiculite and charred rice husk) can be used to obtain nematode-free stock. It is important regularly to treat seedling containers or boxes to maintain hygiene. Where available through (international) trade at relatively low prices, healthy, certified seed or nursery material can reduce the need for farmers to produce their own pest-free material. Practices that are increasingly adopted are the use of tissue-culture propagation techniques, e.g. for ornamentals and bananas, and the floatation tray method as applied for tobacco and flower seedlings in Africa. At the same time, such systems also provide the opportunity to deliver optimally seedling protectants including beneficial microorganisms.

Where nematode-free propagation material is not guaranteed or available, disinfection of (potentially) infected material can provide a solution. Heat treatment and mechanical methods to separate infected from healthy seeds are widely practised methods to obtain nematode-free plants. However, if planting material is not treated or infected soil is used for seedling production before distribution, the practice can result in greater distribution of nematode (and other) pests, so caution is required.

13.7.2. Heat treatment

Hot-water treatment (therapy) is used to disinfest vegetatively propagated planting material such as tubers, bulbs, rhizomes, runners, woody rootstocks and also seeds. The practice relies on the application of sufficient heat over a sufficient length of time to prove lethal to nematodes without thermal injury to the planting material. The greater the difference in the thermal sensitivity between the plant host and the nematode pest, the greater is the potential success for decontamination. In Europe, the use of hot-water treatment for the management of *Ditylenchus dipsaci* led to the undoubted improvement in ornamental bulb health. In combination with infected root and corm tissue removal (paring), banana and plantain nematodes (*R. similis, Helicotylenchus multicinctus*, *Pratylenchus* spp. and *Meloidogyne* spp.) have been successfully managed by dipping rhizomes (suckers) in water at 53 - 55°C for 20 min. Other tuber, corm or bulb crops have been similarly successfully treated. Where fuel is scarce or costly, or temperature and timing regulation is problematic, such as in many subsistence-farming systems, this approach may not be practical. However, a simplified technique using local materials (e.g. empty oil drums) has been adapted and uses boiling water in which pared banana suckers are immersed for short periods of only 30 s (Coyne *et al*., 2010; Fig. 13.5). Hot-water treatment can also be further improved with pre-treatments, such as inducing cold-hardiness by storing at low temperature (e.g. roses), pre-warming (e.g. strawberry plants), pre-soaking (e.g. rice seeds) and/or immersing in cold water after heat exposure (e.g. grapevine rootings). Pre-soaking with solutions of hydrogen peroxide, sodium chloride or other salts can further improve the process. If conditions are unfavourable for efficient drying, the treatment of large volumes of seed may be inadvisable, as fungal contamination or premature germination may result. Spreading out dry seed under intense solar radiation can also help reduce nematode seed infestations, e.g. *Aphelenchoides besseyi* in rice.

When using hot-water treatment for nematode decontamination, it is necessary to test and modify the technique to suit individual circumstances. Different crops, and even cultivars, differ in their sensitivity to heat, which may additionally differ depending on their storage or growing conditions.

13.7.3. Mechanical methods

In the case of *Anguina tritici*, which infects wheat species (*Triticum* spp.), including emmer and spelt but also rye (*Secale cereale)*, the infected seeds that turned into galls (cockles) can be separated from healthy seed stocks by weight differential; as the galls are less dense, they can be winnowed or separated by floatation in salt solutions. They can also be separated using sieves, relying on size differences.

Seed-borne nematodes that may infect other crops include *A. agrostis* and *A. funesta* on grasses, e.g. on bentgrass (*Agrostis* spp.) in the USA, as well as *A. arachidis* and *D. africanus* affecting groundnut in Africa. These seeds may also be cleaned using weight or size differentials of infested seed, or simply by visual selection of the infected distorted or discoloured seed (Brown and Kerry, 1987).

13.8. Sanitation

Approaches that limit the build-up or survival of nematode populations should be practised wherever feasible. Simple sanitation measures to reduce nematode movement between sites on implements or through irrigation or waste products are probably not a foremost consideration in most circumstances, but are of major importance as they contribute considerably to the spread and build-up of nematode populations. However, such practices can be time-consuming and farmers may be reluctant to implement them if the objective is not clearly understood.

Post-harvest sanitation and physical destruction of plant debris, such as straw and stubble burning has traditionally played an important role in the management of some seed and stem nematodes such as *A. agrostis, A. besseyi, A. tritici* and *D. angustus*. Burning reduces the return of nematode inoculum to the soil after a susceptible crop. This is also the case for the traditional post-harvest burning of uprooted tobacco plants in Malawi as a means of managing *Meloidogyne* spp. However, environmental protection acts now restrict this practice in some parts of the world, limiting the use of this method for sanitation purposes.

In intensive potato production areas in Europe, outgraded potato tubers left in the field after harvest can sprout and grow among plants of the next crop in the rotation, thus enabling potato cyst nematodes (*Globodera* spp.) to reproduce despite the strict rotation scheme. Removal, most often by destruction using selective herbicides, prevents build-up of cyst populations. In the intensive potato industry, tare soil and soil adhering to tubers are transported by trucks over long distances, thereby distributing potato-cyst nematodes. Washing potato tubers free of soil has proven to be effective in the USA for many years. As this practice is not always possible, desinfestation of soil is receiving more attention, especially in view of international trade.

For two nematode species, *Bursaphelenchus xylophilus* and *B. cocophilus,* which affect pine and palm trees, respectively, the treatment of wood and wood products is a key measure towards reducing their spread. *Bursaphelenchus xylophilus* can be controlled in timber and wood chips by proper heating in kilns before removal from infested sites, a practice already required for international trade of selected wood products as a preventive measure. Pesticides can also be used to control *Bursaphelenchus* spp., provided the use of these chemicals is not prohibited. A principal method of containment and management is the removal and destruction by burning dead and dying trees.

13.9. Physical Soil Treatments

Managing nematode population densities in the soil to levels below the damage thresholds can be achieved by preventing nematode multiplication or survival, or by killing them directly. Over previous decades soil desinfestation has relied heavily on broad-spectrum biocides, at least in developed countries (Chapter 16). Alternative methods for disinfesting or sterilizing soil rely on physical methods.

13.9.1. Dry heat

The use of fire is not the most efficient way to apply heat for disinfesting soil. The fuel requirements to permit lethal heat levels to penetrate sufficiently deep are uneconomical in most circumstances. However, it may be practical and effective for small areas of land, such as in the treatment of seedling nurseries. The use of slow burning rice husks in Asia has provided nematode-free soil for use in rice and vegetable nurseries. Pre-drenching the soil, before placing fuel and igniting, further improves the depth of penetration of lethal heat and the general efficiency of the process. Dry heat can be used to treat bulk and container soil using various heating devices, e.g. metal plates heated by electricity placed at regular spacing in the soil. Depending on the method and soil type, changes in the physical and chemical nature of treated soil can cause unsatisfactory plant growth.

The use of microwave radiation for treatment of soil against nematodes has been demonstrated for *H. schachtii* and *Rotyenchulus reniformis*, but this method is still not applied widely as it appears to be an impractical method for field soil. Research continues however, for alternatives to synthetic pesticides that may prove suitable for small volumes of soil or for nursery situations.

13.9.2. Steam

Steam heat has long been applied in heated glasshouses as an effective, if costly, means of soil sterilization. Soil type and prior soil tillage affect heat penetration and efficacy, as does water absorption capability. Dry soil has a greater capacity to absorb condensed water and to a greater depth than wet soil. Steam can be applied by pumping through a network of underground, perforated pipes (about 60 cm depth). Even distribution of heat through the soil, especially the surface layers, is improved by covering the soil with plastic sheeting. Blowing steam under plastic sheeting (sheet steaming), which has been anchored at the edges, provides surface soil sterilization. Sheet steaming, combined with buried pipes can create ‘negative pressure steaming’, a more energy efficient and effective method that draws the steam through the soil using an extractor fan (Maas, 1987). The main drawback to the use of steam is cost. Treated soil should be set aside for some time to permit the soil to stabilize as the release of phytotoxic chemicals (nitrite, ammonia) and change in pH may affect plant growth. For less commercial systems, steam can still be effectively employed for the use of small volumes of soil, such as for use in nurseries. Containers, such as used oil drums, can be semi-filled with water, placed over a heat source and adapted to deliver steam beneath the soil via a pipe attached to the steam outlet on the drum. Plastic sheeting can be used to contain the steam in the soil being treated.

13.9.3. Solar heat

Solar radiation has been employed effectively to disinfest soil of nematodes, primarily in hot climates and for relatively shallow depths of soil (Gaur and Perry, 1991). Moistened soil covered with polyethylene sheeting, with the edges anchored, will significantly reduce nematode populations (Fig. 13.6). Moisture is necessary as this promotes biological activity, improving efficacy. This system has been variously termed plastic-, polythene-, polyethylene-mulching or tarping, solar heating, solar pasteurization or soil solarisation, the latter term being the more generally accepted. The effect reduces with depth, but solarization for at least 4-6 weeks will increase soil temperatures to 35-50°C to depths of up to about 30 cm, depending on soil type and prior tillage. Results can be optimised by using double layers of polyethylene, thin (25-30 µm), transparent as opposed to black sheeting, and using solarisation during periods of highest solar intensity. Thinner sheeting tends to be more effective but less durable and more easily damaged. Larger areas are also more practical to treat, as soil heating is less effective near the edges of the covered area. The length of time required for effective solarisation is a great limitation and the method is most suited to nursery beds and in glasshouses rather than large field areas. Also, in areas of intensive use, the disposal of the large quantities of plastic sheeting can be a problem. Excellent nematode control in soil for use in potting composts, to raise seedlings or rooting cuttings, can be achieved for small volumes of soil, which are moistened, contained in sealed plastic bags and placed on a suitable surface in direct sunshine for two weeks. An additional benefit of solarisation is the control of soil-borne pathogens and weeds.

13.9.4. Flooding

Areas of land following natural inundation are mostly free of plant-parasitic nematodes. Areas such as river flood plains, riverbanks and seasonally flooded lakes, have long been exploited for crop production, primarily to take advantage of fresh sediment deposits. Extended periods of flooding provide almost nematode-free conditions. Notable exceptions are the rice root nematodes, *Hirschmanniella* spp., and the foliar nematodes, *A. besseyi* and *D. angustus*. Some species of *Tylenchorhynchus, Meloidogyne, Helicotylenchus, Heterodera, Rotylenchus, Pratylenchus, Paratylenchus, Hemicycliophora, Xiphinema* and Criconematidae are also known to survive periods of flooding. If small in area, flooded sites may be used for nursery purposes or, alternatively, the soil may be removed for use in a nursery sited elsewhere. Artificially flooded areas, such as rice paddies, can also provide nematode-free conditions for post-rice crops that are non-hosts for the rice nematodes. Rice-vegetable and rice-wheat production systems tend to be quite sustainable, despite the presence of nematode pests, including species of *Meloidogyne*. This is partly a consequence of the nematode suppressiveness of soils flooded for the rice crop. A disadvantage of flooding is that the field cannot be used to grow crops, and so provide income, for an extended period of time. Fields also need to be non-sloping. As the use of nematicides becomes more restricted, the practice of flooding has gained more attention in high-value crops where quarantine regulations require zero levels of certain nematode species, e.g. flower bulbs fields in The Netherlands are kept inundated for 17 weeks to reduce *D. dipsaci* to undetectable levels.

13.9.5. Anaerobic soil disinfestation

A technique called anaerobic soil disinfestation (ASD) - also called biological soil disinfestation (BST) - has been developed as an alternative to the use of methyl bromide. ASD consists of incorporating copious quantities of organic matter into the soil, followed by irrigation to obtain a moisture level above field capacity and carefully sealing the amended soil with impermeable polyethylene sheeting for several weeks. The decomposing organic matter (e.g. rice husks, grass or crop by-products) and the wet conditions result in anaerobic conditions while several toxic gaseous compounds are produced. The method requires sufficiently high temperatures and careful sealing to trap the gasses and establish the anaerobic conditions necessary for killing soil pathogens, including nematodes. ASD is applied on sandy soils for strawberrys and asparagus production in The Netherlands and California. The method still needs optimization, but is promising (Thoden et al., 2011).

13.10. Biologically Based Practices

13.10.1. Crop rotation

Seasonal rotation on the same area of land with different crops remains one of the most important methods of nematode management. Rotation with crops, which have different nutritional demands on the soil and have different pest problems, has obvious benefits to the maintenance of the system. Supposedly, the Incas practised crop rotation in response to damage caused by the host-specific potato cyst nematodes. However, intensification of cropping systems and the success of pesticides has seen reduced reliance on crop rotation in modern agriculture. The basic premise of crop rotation is to distance the time between susceptible hosts to the same nematode species using resistant, poor or inhibitory hosts, in order that population densities do not increase to damaging levels, or decline below damage thresholds before the next fully susceptible crop is grown. The number of cropping sequences or the period between susceptible hosts can depend on, in particular, the host status of the rotation crops (and cultivars), the nematode species and the nematode population density at harvest of the last susceptible crop. A rotation of at least 7 years between potato crops was traditionally necessary to prevent losses due to *G. rostochiensis* and *G. pallida* in Europe but with the integrated use of nematicides and cultivars resistant to *G. rostochiensis*, this has since been shortened (Phillips and Trudgill, 1998). While crop rotation is widely practised and is environmentally appealing, it has important limitations. The occurrence of nematode communities containing multiple pests or polyphagous species with wide host ranges, such as some species of *Meloidogyne* or *Pratylenchus*, limits the potential of using acceptable non-host crops for the rotation. Rotation crops may also facilitate the increase of alternative nematode pest species. The degree of control is dependent on the level of resistance of the rotation crops and the length of the rotation cycle, which may be too long to be acceptable, especially for specialist producers in intensive production systems and for subsistence farmers with limited land. Further difficulties arise where the non-host crops have no local market or are of limited value. Correct nematode identification and knowledge of the host range and cultivar susceptibility, therefore, determine the introduction of successful rotations. *Meloidogyne* spp. as a group presents the most formidable challenge to the implementation of successful rotations because of their broad host range. Accurate identification of species present is essential but the development of ‘resistance-breaking’ populations, the emergence of virulent strains/races or pathotypes and communities of multiple species of *Meloidogyne*, all pose a challenge. Poor control of weeds, which host polyphagous nematode pests, will also reduce the effectiveness of crop rotations. Nevertheless, crop rotation as a nematode management strategy can be employed efficiently and can provide an essential measure in integrated pest management programmes.

Heterodera glycines, *G. rostochiensis, D. dipsaci* and even some species of *Meloidogyne* have limited host ranges amongst crop species or, alternatively, occur in situations where they tend to be the dominant nematode species. They provide examples where crop rotation has provided effective nematode management. *Heterodera glycines* has few alternative host crops and cultivation of non-hosts such as corn (maize) and groundnut for 2 years can be sufficient to provide a full yield of soybean. This can be further reduced, or the number of consecutive soybean crops increased, through the integration of resistant cultivars (see Chapter 14). In California, successful management of *M. naasi* on barley has been accomplished through barley-oat rotations. The development of cultivars with resistance to key nematode pests continues to improve the flexibility of nematode management programmes based on crop rotation.

13.10.2. Trap cropping

Certain situations may accommodate the implementation of trap cropping, where a highly susceptible host is planted and grown for sufficient time to permit nematode invasion and development, but not to complete its life cycle. This strategy is primarily employed for sedentary endoparasitic nematode management. The trap crop must be physically removed or destroyed (with herbicide or ploughed in) before the nematodes reproduce.

An alternative approach involves the use of resistant crops or cultivars, which stimulate nematode activity and/or support nematode invasion but do not permit completion of the life cycle. Such plants are also termed trap crops. Examples of resistant crops that do attract nematodes are sun hemp (*Crotalaria spectabilis*) for *Meloidogyne* spp., *Sesbania rostrata* for *Hirschmanniella oryzae* in rice and *Solanum sisymbriifolium* for the control of *Globodera* spp. on potato in Europe (see Chapter 8).

The trap crop is preferably planted quite densely so that roots and root diffusates reach as many nematodes as possible. Success depends upon proper planting techniques, precise timing and total crop destruction in the case of susceptible crops. Trap cropping is therefore rather costly and inconvenient but can be a very effective tool for nematode management.

13.10. Antagonistic Plants

Plants with nematode antagonistic properties, due to root diffusates, can be used in rotation or as intercrops with susceptible crops against certain nematode species. Species of marigold (*Tagetes erectus, T. patula* and *T. minuta*) are good examples, which successfully reduce populations of *Pratylenchus* spp. and *Meloidogyne* spp. as well as other species. The mode of action of *Tagetes* spp. is attributed to alpha-tertienyl, a very toxic compound contained in the root cells, but also simply to its non-host status for several nematode species, although some Trichodoridae are reported to increase on marigold.

The value of the crop and of the antagonistic plants determine the suitability of this approach, although antagonistic crops with a potential market, such as asparagus (*Asparagus officinalis*), will undoubtedly improve the acceptability. For example, use of *Tagetes* spp. for nematode management has increased with its increased use as a source of xanthophylls for food colourants.

13.10.4. Cover crops

Numerous examples of plants that are antagonistic, resistant, suppressive or detrimental to the population development of plant-parasitic nematodes are available (see Whitehead, 1998; Luc *et al*., 2005). However, many such plants often have no direct commercial value, limiting their appeal, compared with those that have an alternative use. Additional functions may include use as cover crops, for soil conservation during the off-season (winter or dry period), or as forage for livestock (e.g. joint vetch (*Aeschynomene* spp.), hairy indigo (*Indigofera hirsute*), stylo (*Stylosanthes* spp.)). Leguminous crops contribute to soil nitrogen availability, whilst crops that produce extensive foliage may be ploughed in as a green manure. Cover crops with nematode suppressive qualities vary with geographical location and target nematode species. Noteworthy examples include *Aeschynomene* spp., horsebean (*Canavalia ensiformis*), butterfly pea (*Centrosema pubescens*), *Crotalaria* spp., kudzu (*Pueraria phaseoloides*), castor (*Ricinus communis*) and particularly velvetbean (*Mucuna pruriens* and *M. deeringiana*). In tropical regions, grasses and cereals are generally poor hosts of *Meloidogyne* spp. and are often successful in reducing *M. javanica* and *M. incognita*. Additionally, when ploughed in, some crops produce or release nematotoxic compounds upon decomposition. Brassica crops such as rapeseed (*Brassica napus*) and mustard (*B. campestris*), contain glucosinolates, which become hydrolysed to the volatile isothiocyanate and other products with broad biocidal activity. These act as biofumigants following incorporation of brassicaceous residues into the soil, reducing soil-borne pathogens including plant-parasitic nematodes. Brassica crops and cultivars vary in their glucosinolate content and their effects on nematodes vary according to the physiological stage of the nematode and the environmental conditions. Their role in reducing pathogen densities is also attributed to merely adding organic matter to the soil, thereby stimulating diverse soil microbial activity, which in turn can have a positive effect on plant growth while reducing the share of plant pathogens.

Taking advantage of the off-season to grow a poor host as a cover crop has numerous merits for the farmer. However, if this period coincides with a period of natural nematode dormancy (see Chapter 8) or low activity, only limited nematode management may be achieved.

13.10.5. Fallow

The term black fallow is used when no vegetation is allowed to grow on land, whereas fallow refers more to a period without agricultural cropping, but where weeds can still maintain or even increase nematode populations. Keeping land free of vegetation through frequent tillage or herbicide application can reduce nematode populations primarily through starvation, although desiccation and exposure to solar heat may contribute. In general, the practice is not particularly attractive due to the increased risk of soil erosion and loss of production during the period of black fallow.

In traditional subsistence cropping systems in developing countries, small-holders rely on natural fallow periods for several seasons following crop production, for restoration of soil fertility, maintenance of soil structure and suppression of pests. However, this practice can be maintained only where sufficient land is available. Intensification of cropping practices and rising human populations increasingly limit the extent of this practice.

13.11. Amendments

Application to soil of fertilisers or organic matter is a readily accepted practice for improving crop production, primarily with regard to improving soil fertility and structure but which can also lead to reduced plant-parasitic nematode population densities.

13.11.1. Organic matter

Amending soil with various sources of organic matter (Table 13.1) has led to reduced nematode problems, either by reducing population levels or by increasing yields without affecting populations (Widmer *et al.*, 2002; Thoden *et al.*, 2011). The specific mechanisms of control are not clearly understood. Nematode suppression is certainly attributable to increased saprophytic and antagonistic soil biota activity following application of organic matter. It is also recognized that crops are less stressed and more tolerant of nematode parasitism when grown with mulches than in less favourable conditions. The release and consequent build up of organic acids, phenolic compounds, ammonia or other compounds to concentrations toxic to nematodes are also prime factors in the nematicidal activity of different soil amendments. Numerous nematode genera are reported affected, although most studies deal with species of *Meloidogyne*. Application of oilseed waste (cakes), such as castor, neem (also known as margosa) (*Azadirachta indica*), cotton (*Gossypium* spp.), groundnut and mustard, amongst others, appear particularly effective at reducing nematode population levels. Waste crop by-products, such as sawdust, fruit pulp, coffee husk, oil palm debris and molasses, are also attractive amendments. Although such waste products tend to be inexpensive, they are often only locally available and require transportation to the field. Consequently, their practical use is of limited value for widespread field implementation. However, locally they can offer an effective means of nematode management and soil fertility improvement. Amendment with animal waste products such as manures, bone-meal and chitin can also lead to reduced nematode populations. The addition of crustacean chitin has proved highly effective, leading to the registration of commercial products. Chicken manure also appears particularly effective and, as with chitin, its activity probably depends on the release and build-up of nematotoxic levels of ammonia. In some banana production systems mulching with a range of organic material has led to reduced damage by *R. similis*, caused by several factors, including suppressing temperature increases, which would otherwise be more optimal for nematode multiplication. Applying animal manure or cultivating a green manure crop is a regular agricultural practice. The additional benefit of nematode management may thus be an important and useful supplementary gain. However, although the mechanisms surrounding the reduction of nematode populations or damage appear complex, in general, large quantities of material are necessary to be effective. Therefore, the material must be locally available and inexpensive.

In addition to amending soil with plant material, aqueous extracts or leachates from plants with nematicidal properties has received substantial attention. Commonly termed a botanical pesticide, the principle exploits nematicidal compounds from certain plants, which are applied as a pesticide following extraction. Many plants with nematicidal properties are also applied as organic amendments, in the form of leaves, bark or pounded seeds, for example to the base of the planting hole or as a mulch. Neem (*A. indica*) is perhaps the most studied, with some convincing results against various nematode species when applied either as a mulch, dip or drench. Other notable examples include *Tagetes* spp., *Crotalaria* spp. and lemon grass (*Cymbopogon flexuosus*), whilst some seaweeds are less well known but produce a range of bioactive compounds.

13.11.2. Non-organic

Various levels of nematode control following application of mineral fertilizers have been observed. Applications of certain fertilizers may be toxic to nematodes or suppress their multiplication and damage through changes in host nutrition. The interactions between N, P and K availability and nematode populations and/or damage has probably received most attention, especially K, which is also understood to have a general balancing effect on N and P. However, results are often contradictory or highly variable in the levels of control obtained

(Coyne *et al*., 2004). Indeed, this may depend on several factors such as the fertilizer type and rate of application, the minerals applied and their chemical formulation. However, differences in biotic and abiotic factors between sites, in climate and environmental factors, which affect nematode population dynamics, undoubtedly compound the influence of mineral availability on nematodes. Moreover, the magnitude and complexity of the probable interactions makes it difficult to provide general recommendations on the use of mineral fertilisers for nematode management.

13.12. Time of Planting

Planting to avoid periods of peak nematode activity can be exploited in certain circumstances to reduce nematode damage. Crops planted during periods when temperatures are sub-optimal for nematode development can enable seedlings to be sufficiently advanced to withstand nematode attack. Crops may still be affected but, due to delayed nematode maturation, peak activity may occur too late in the crop cycle to result in heavy yield losses. Autumn sown, as opposed to spring sown, cereals in Europe suffer less yield losses to *H. avenae*; carrots are sown late in California to avoid *M. incognita* damage. Care should be taken, however, not to cause a shift in nematode species that are better adapted to the time of planting. Early planting of potatoes in North-West Europe to avoid damage by *G. rostochiensis*, contributed to the shift towards *G. pallida*, the potato cyst species that hatches at colder temperatures.

13.13. Other Control Practices

Various other practices can also incidentally affect nematode multiplication. For example, weeding may affect nematode densities either positively or negatively, depending on whether the dominant weeds are nematode hosts, antagonists or trap crops. If dominant weeds are a preferred host of key nematodes, their removal may result in increased nematode damage to the crop due to removal of the main host, or less damage to the crop if the weeds can be exploited as trap crops. The level of nematode attack on fruit trees can also be related to the timing of pruning. Knowledge of such interactions may help growers improve their cropping practices simply by adjusting the timing of their regular activities. In other cases, nematode damage can influence breeding criteria for particular traits, such as shallow rooting systems in citrus trees, which may be more conveniently treated with nematicides than deep-rooted trees.