Chapter 23
Biological Control of Plant-Parasitic Nematodes: Towards Understanding Field Variation Through Molecular Mechanisms

Keith G. Davies and Yitzhak Spiegel

23.1 Introduction

Biological control is a subject that covers a huge area of application, bugs to control other bugs for application in medical, animal husbandry and agricultural situations. Stirling (1991) defined biological control of plant- parasitic nematodes as, 'A reduction of nematode populations which is accomplished through the action of living organisms other than nematode-resistant plants, which occurs naturally or through the manipulation of the environment or the introduction of antagonists'. In all these situations the underlying biology rests on the generic science pertaining to an interaction of one organism with another organism, either directly or indirectly, and it is sufficiently well understood that it can be used and be exploited to reduce an unwanted organism, in this case a nematode pest, to a number whereby it no longer has a detrimental effect on crop yield.

23.1.1 Historical Context

Prior to the use of nematicides, biological control was always viewed as a possible way to control plant-parasitic nematodes and as early as 1920 Cobb had suggested the use of predatory nematodes in sugar beet fields to control *Heterodera schachtii* (Cobb 1920). Further research suggested this approach was not economically viable (Thorne 1927). Several years later the focus changed to the use of predatory fungi and in particular the use of nematode trapping fungi (Linford 1937; Linford et al. 1938). However, these promising results were eclipsed by the spectacular results that began to be obtained by the newly developed nematicides (Stirling 1991). Throughout the later part of the twentieth century plant-parasitic nematodes have successfully been controlled by the use of these compounds, which were

K. G. Davies (✉)
Plant Pathology and Microbiology Department, Rothamsted Research, Harpenden, AL5 4PT, UK
e-mail: keith.davies@bbsrc.ac.uk

J. Jones et al. (eds.), *Genomics and Molecular Genetics of Plant-Nematode Interactions,*
DOI 10.1007/978-94-007-0434-3_23,

manufactured through the petro-chemical industry. More recently, with the increasing recognition of their toxicity and their harmful effects to both the environment and human health, legislation has been implemented to reduce their use. Although biological control has always tended to be in the shadow of plant resistance as an alternative to chemical control, it is again back on the agenda.

The focus of this renewed research effort into biological control was built on the observation that although mono-cropping can lead to an increase in plant-parasitic nematodes, with a concomitant reduction in crop yield, continued mono-cropping leads to the production of nematode suppressive soils (Gair et al. 1969). Subsequent research has shown that predatory fungi were responsible for this suppression (Kerry 1975; Kerry and Crump 1977; Kerry et al. 1982). The recognition that parasites were responsible for the development of nematode suppressive soils lead to the 'classical' approach to biological control where parasites were identified in nematode suppressive soils, mass produced and introduced into areas were nematode pests were abundant (Stirling 1991). Despite the best efforts of biological control scientists over the last 20–30 years, and although impressive results have been obtained using this approach, generally speaking this has not produced a robust method of nematode control which is consistent and broadly applicable. To date, there are relatively few commercially available products (Whipps and Davies 2000; Hallmann et al. 2009).

23.1.2 Technical Developments and Twenty-First Century Science

During the last decades of the twentieth century most research groups focused on attempting to isolate soil microorganisms, mainly fungi and bacteria, without devoting any special concern as to the mode-of-action of these potential microbial control agents. Early research that explored the mechanistic interactions between plant-parasitic nematodes and trapping fungi initially started out as fundamentally morphological in nature. This research later developed to examine biochemical aspects of infection processes and this approach continues today with an emphasis on molecular and genomic aspects (Tunlid and Ahrén 2010; Davies and Spiegel 2010). Similar trends can be seen within other research groups and this emphasises the interaction between the growth of biological control knowledge through research and its dependent relationship with technological development. For example, initial morphological studies exploited the use of light- and electron microscopy, which in turn expanded into investigations on 'ecological' aspects based on observations made using microbiological techniques such as selective media for the quantification of microbial populations in soil (Kerry and Hirsch 2010). The first decade of the twenty-first Century witnessed the use of a combination of approaches (molecular biology, biochemistry, genetics/genomic and developmental biology) aimed at getting a deeper understanding of such interactions (Davies and Spiegel 2010) and the challenge is therefore to bring a coherence to these approaches in an effort to develop robust control strategies that can work in the field situation.

23.2 Ecological Context

The most economically important plant-parasitic nematodes are root parasites of crops, where they inhabit the water films on soil particles and can feed on plant roots. This environment is highly fragmented and subject to continual wetting and drying. Because nematode movement is only possible between a water tension of 4.2 (a level at which plants are wilting) and 4.45 pF (Jones and Jones 1974), the hydraulic properties of the soil are critical and these are continually changing both spatially and temporally (Avendano et al. 2004).

23.2.1 The Soil as a Heterogeneous Environment

As large amounts of carbon are exuded by plant roots (Bekku et al. 1997) this carbon source is an important driver for determining the population dynamics of organisms occurring in the rhizosphere where nematodes reside as part of the community. Roots that permeate the soil are not homogeneous in their distribution but exploit nutrient rich patches which lead to changes in root morphology (Hodge 2006; Watt et al. 2006). Plant-parasitic nematodes, which are sensitive to the changing chemical properties of the rhizosphere, use these to locate plant host roots and therefore they are also patchily distributed (Costa et al. 2010).

23.2.2 Food-Webs and Multitrophic Interactions

Plant-parasitic nematodes form a part of a below-ground tritrophic interaction between plant roots and the soil/root microbial community in which the populations of phytonematodes are controlled by a number of different processes that include bottom up, horizontal, and top down control.

23.2.2.1 Bottom up Control

Bottom up control is where a population of nematodes is determined by the level of nutrients available. In the case of plant parasitic nematodes, which are obligate parasites of plants, this has involved a co-evolving arms race between host plants and parasitic nematodes. It is interesting that different species of plant-parasitic nematodes have adopted different life-cycle strategies that arguably form an evolutionary continuum from migratory ectoparasites, through to migratory endoparasites and on to sedentary endoparasites (Wood 1973; Yeates et al. 1993) exhibiting increasing specialisation. Interestingly not all plants are equally susceptible to nematode parasitism and different plants exhibit differing degrees of resistance and/or tolerance (Cook

and Starr 2006; Trudgill 1991) and of the most economically important nematodes, the cyst nematodes are regarded as considerably more host specific than the polyphagous root-knot nematodes. However, due care needs to be taken when investigating such parameters because the susceptibility of a particular plant to a particular nematode may be due to endophic fungi and or arbuscular mycorrhizal fungi (Hallmann and Sikora 2010) that may be playing an important role in bottom up control. The role of rhizobia on legumes and their ability to reduce nematode populations is much less clear with reports of some strains of rhizobia capable of inducing resistance in the root against plant-parasitic nematodes (Reitz et al. 2000; Mitra et al. 2004).

23.2.2.2 Horizontal Control

The maximum number of nematodes that can be sustained on a given root system represents its carrying capacity and the population increase can be modelled as a curve in which there is increased intra-specific competition for the available niches present on the root as the number of individual nematodes increases (Varley et al. 1973). Two species of nematode that reproduce continually and have overlapping ecological niches would also be subjected to intra-specific competition at low population densities. However, as populations of each species became larger they would increasingly be subjected to inter-specific competition, the outcome of which would be that one nematode species would be reducing the number of the other species (McSorley and Duncan 2004; Van den Berg and Rossing 2005). This control of one species of nematode by another represents horizontal control (Van der Putten et al. 2001). Competition experiments have been undertaken to evaluate the extent to which horizontal control plays a role in determining the population densities of *Meloidogyne maritima, Heterodera arenaria* and *Pratylenchus penetrans* in marram grass on costal sand dunes and it appeared that the outcomes were mediated by the host plant (Brinkman et al. 2005; de la Pena et al. 2008).

23.2.2.3 Top Down Control

The use of top down control is the strategy employed by most scientists envisaging the use of inundation of a selected natural enemy, usually a fungus or a bacterium, to control a particular plant-parasitic nematode. This method is based on the isolation of a nematode natural enemy that is parasitic on a particular nematode pest from a field that is nematode suppressive, mass produced and then deployed back to a field where the nematode is the cause of yield decline. Although this method has proved useful in pot tests and glasshouse studies, scaling up this approach to the field scale has been problematic leading to variable results. Analysis of the problem from an ecological perspective may therefore provide useful insights.

Field populations of nematodes usually occur as mixtures, both mixtures of different species and mixtures of different sub-species. Therefore in a nematode suppressive soil one would imagine that the natural microbial enemies of such a com-

munity would also contain a mixture of different species of microbial enemies and sub-species of microbial enemies. What follows *a priori* from the Red Queen Hypothesis (Van Valen 1973, 1976) suggests that a co-evolutionary arms race between hosts and parasites will lead to genetic diversity in which hosts are driven to produce genetic combinations which decrease parasite virulence, whereas parasites are driven to produce genetic combinations to maintain virulence. The results of such an arms race over extended time would be to allow infection and multiplication of the parasite without a detrimental effect on the host (Ebert and Hamilton 1996).

Co-evolutionary mathematical models predict that parasites that have a broad host range will tend to diverge, generating sub-populations with different host specializations. Because of the smaller size and quicker generation times of microbial parasites over their hosts each microbial subpopulation will co-evolve faster with its host, than a generalist population can evolve with different and variable hosts (Kawecki 1998). Therefore, at the initiation of such a host-parasitic arms race, it is not surprising that boom and bust population dynamics are the norm, and that the success of any selected microbial enemy from a particular pest nematode, deployed back into a field will produce inconsistent results. Investigations looking at microbial diversity and parasitism seem to support this view (see Sect. 23.4).

23.3 Molecular Approaches for Assessing Field Biodiversity

To gain insights into nematode suppression for the development of biological control strategies it is becoming increasingly obvious that knowledge of the diversity of the nematodes and their natural enemies, together with their temporal and spatial distribution, will play a key role. Conventional methods of identifying and quantifying both nematodes and their microbial enemies is time consuming and labour intensive and requires a high level of technical expertise, which, when all taken together, compromises the number of samples that can be processed (Costa et al. 2010). In addition, because of the heterogeneous spatial distribution of nematodes and their microbial enemies, and the limitations of soil sampling, most sampling techniques usually average the distribution of organisms and vary in their efficiency of their extraction (Ettema and Wardle 2002; McSorley and Frederick 2004). The population dynamics of host-parasite interactions occurring in the soil are therefore notoriously difficult to ascertain. The ability to quantify relationships between hosts and their parasites will be important in the development of biological control strategies and new molecular approaches have an important role to play.

23.3.1 Antibodies

Antibodies are a class of globular proteins produced by the lymphatic system of animals in response to its invasion by a foreign compound or organism. The function

of antibodies is to label these foreign bodies for destruction by the immune system. Antibody binding is very specific and this specificity can be used as a tool in the identification and diagnosis of pests and other organisms (Clark 1994). Antibodies have been shown to be useful in the identification and quantification of both plant-parasitic nematodes and their microbial enemies (Davies 1994). Antibodies can be made with variable degrees of specificity and theoretically can be made into a diagnostic tool for any level of molecular operational taxonomic unit (MOTU) required. Once produced, they are generally easy to use in a number of different formats from dip stick type assays and enzyme linked immune-assays through to fluorescence microscopy (Clark 1994). They can be used qualitatively as well as quantitatively and are therefore highly flexible (Davies et al. 1996). Monoclonal antibodies have been used to differentiate species of both root-knot nematodes (Davies and Lander 1992) and potato cyst nematode (Robinson et al. 1993) and can be used to quantify nematodes in soil samples (Davies and Carter 1995). Antibodies have also been successful in monitoring the nematophagus fungus *Pochonia chlamydosporia* and the bacterial parasite *Pasteuria penetrans* in the rhizosphere using immunofluorescence microscopy (Hirsch et al. 2001; Costa et al. 2006).

23.3.2 Phospholipid Fatty Acid Analysis (PFLA)

Fatty acids can be used in the classification and identification of microorganisms (O'Donnell 1994) and the interaction of nematodes with their fungal and bacterial communities has been studied using PFLA profiles (Bardgett et al. 1996; Denton et al. 1999). Changes in PLFA profiles reflect changes in the microbial community structure and are indicative of the biomass of the various groups of bacteria and fungi present (Bardgett et al. 1996). However, although the method is sensitive enough to monitor changes to some different bacterial groups, all fungi are measured by one particular fatty acid and this therefore limits their application (Denton et al. 1999). Thus, although PFLAs have been useful in monitoring changes in the microbial communities in relationship to environmental conditions (O'Donnell et al. 2005), they have limited use in assessing microbial diversity in any detail. However, PFLAs have been influential in studying the interactions and inter-connectedness of different trophic groups of nematode populations in a below ground grassland ecosystem (Bardgett et al. 1999).

23.3.3 DNA

Identification of organisms using DNA-based techniques on a routine basis dates back to the 1980s but was revolutionised by the invention of the Polymerase Chain Reaction (PCR) which relies on enzymatic amplification of a given DNA sequence (Saiki et al. 1985). A PCR-based method called denaturing gradient gel electrophoresis (DGGE) has enabled community fingerprints of large groups of microorgan-

isms to be obtained that provides a measure of their genetic diversity (Muyzer et al. 1993). Originally this technique was applied to bacterial communities in soil by amplifying the 16s rDNA gene and this combined with the sequencing of excised bands from the original gel can provide further taxonomic information (De Mesel et al. 2004). Similar approaches, using 18s rDNA have been applied to fungal communities but their quantification is complicated by the inability of the method to distinguish fungal spores from vegetatively growing hyphal fragments (van Elsas et al. 2000). DGGE analysis has been used to show that plant defence against nematodes was not mediated by changes in the soil microbial community (Wurst et al. 2009). Similar approaches have also been used to study nematode communities (Waite et al. 2003), but, because the number of MOTUs that can be used for describing nematodes is still very small the technique is currently only of limited value (Foucher et al. 2004; Wu et al. 2009).

Another PCR-based approach for characterising nematode communities has been the use of molecular barcodes using the 18s rDNA subunit which are designed to relate to MOTUs (Floyd et al. 2002; Blaxter et al. 2005). Systems are being constructed where it will be possible to use molecular barcodes to obtain sequences that can be used to interrogate online databases to obtain nematode identifications (Powers 2004). Indeed, using these approaches it has been possible not only to identify nematodes but also to construct phylum-wide evolutionary trees (Blaxter et al. 1998; Holterman et al. 2006) that recently have shed light on the evolution of parasitism amongst plant parasitic nematodes (Holterman et al. 2009). Taking these approaches forward it should be possible to elucidate and help understand nematode parasitic interactions such that light will be shed on the functional interactions between the organisms within the rhizosphere community.

23.3.4 Ecological Genomics

Studies of nematode control in their ecological context, particularly top down control which will probably give the most insights into organisms with biological control potential, will need all these molecular approaches to understand the necessary population dynamics that will underpin the development of nematode biological control strategies. The challenge for the biological control scientist is to integrate research in such a way that a model of the rhizosphere community can be developed. From the perspective of controlling plant -parasitic nematodes the new genome sequences (Abad et al. 2008; Opperman et al. 2008), and very shortly those of other nematode species, will be invaluable. This, together with the sequencing of both their plant hosts, and followed by the sequencing of their parasites, will allow the possibility of a multitrophic ecological genomic approach. Rhizospheric ecological studies will then allow the integration of different levels of organisation in which changes in the genetic makeup of a host with its parasites at the population level will be integrated with the specific mechanistic host-parasite interactions at the biochemical and molecular level (Zheng and Dicke 2008).

23.4 Towards Understanding Field Variation Through Molecular Mechanisms: Three Models

If the knowledge gained from sequencing genomes is to be integrated into understanding the population genetics of host-parasitic interactions, the identification of the functional genes involved in compatible and incompatible host—parasite interactions at the molecular level becomes indispensible. In microbial populations, as discussed above, such genes are likely to be involved in arms races that conform to the Red Queen Hypothesis which would lead to the prediction that these genes will have diversified (Van Valen 1973, 1976). Within the context of the soil environment, which we know is likely to be highly heterogeneous with respect to both hosts and parasites, this produces the challenge of species designation or MOTU identification within the context of the metapopulation structure (Fraser et al. 2009) present in the soil habitat. Therefore, understanding the functional mechanisms will be a necessary key to structuring the host-parasite metapopulations in soil and how these interact. This knowledge will be important for the applied biological control scientist to determine in which situations biological control as a management strategy will be successful. Three models are used to explore functional mechanisms of the interactions between potential biological control agents and plant-parasitic nematodes.

23.4.1 Arthrobotrys

Fungi have long been known to be parasitic on nematodes (Barron 1977) and more than 200 species of nematophagous fungi have been described (Tunlid and Ahrén 2010). One group of these are the nematode trapping fungi which, under conditions of limited nutrient availability and in the presence of nematodes, produce specialised organs that can capture nematodes (Barron 1977). The trapping of nematodes by fungi can be broken up into three distinct stages, starting with trap production, followed by recognition and capture and finally penetration and digestion. These three stages overlap to some extent and are not totally separate.

Many trapping fungi produce traps spontaneously, but as early as the 1950s it was shown that “nemin”, a mixture of compounds produced by the nematode, stimulated the formation of traps in *A. conoides* (Pramer and Stoll 1959; Pramer and Kuyama 1963). One particular group of trapping fungi, *Arthrobotrys* spp., have been studied in detail and microscopic studies of *A. oligospora* have shown that the production of traps and subsequent infection of nematodes occurs in a sequence of events, as outlined above, that is completed within 48–60 hours (Dijksterhuis et al. 1994). When a nematode makes contact with a trap a layer of extracellular fibrils that surround the traps become directed perpendicularly to the nematode cuticle and the fungus produces a penetration tube that is thought to be involved in production of hydrolytic enzymes that help to solubilise the cuticle. Then the nematode

becomes paralysed and the penetration tube forms an infection bulb from which hyphae develop and colonise the captured nematode (Tunlid and Ahrén 2010).

23.4.1.1 Lectin-Carbohydrate Interactions

Lectins are proteins that bind carbohydrates and function as recognition molecules (Sharon and Lis 2004). Early experiments based on sugar inhibition and red blood cell (RBC) assays suggested that a carbohydrate present on the surface of the nematode cuticle was interacting with an N-acetygalactosamine (GalNac)-specific lectin present on the surface of the traps in *A. oligospora* (Nordbring-Hertz and Mattiasson 1979). This specificity was confirmed with the finding that Type A RBCs, which contain a terminal GalNac, bound more easily than did Type B and O (Borrebaeck et al. 1984; Premachandran and Pramer 1984). However, the situation is complex, with different species of fungi revealing different binding specificities (Nordbring-Hertz and Chet 1988). Purification of these lectins (Rosén et al. 1992), and more recently the development of specific deletion mutants to see if they played and important role in nematode infection processes (Tunlid et al. 1999; Balogh et al. 2003), suggests they are part of a growing family of lectins that are specific to fungi (Tunlid and Ahrén 2010).

23.4.1.2 *Caenorhabdits elegans*, Genomics and Innate Immunity

Caenorhabditis elegans is a free-living nematode that has been used as a model organism for studying aspects of developmental biology and was the first multicellular animal to be sequenced. *Caenorhabditis elegans* is currently being used to study aspects of pathogenesis and innate immunity (Millet and Ewbank 2004; Darby 2005; Gravato-Nobre and Hodgkin 2005; Fuchs and Mylonakis 2006). The production of strains of *C. elegans* with cuticles that have different surface properties makes it particularly amenable as an experimental tool for dissecting the interactions between the cuticle and microbial pathogens. Many bacteria and fungi have been shown to be pathogens of *C. elegans* (Hodgkin and Partridge 2008). *Arthrobotrys* spp. are regarded as predators of *C. elegans* and bioassays have shown that these nematode trapping fungi differ in their ability to trap mutants of *C. elegans* that show altered lectin staining to the cuticle surface (Mendoza et al. 1999).

More recently, in a study of the infection of *C. elegans* with *Monacrosporium haptotylum*, the most highly up-regulated fungal gene was a subtilisin that was similar in sequence and expression profile to *PII* of *A. oligospora* and designated *spr1* (Fekete et al. 2008). However, another subtilisin *spr2*, although up-regulated at one hour, was then subsequently down- regulated at four and 16 hours only to be up-regulated again at 24 hours when the fungus was digesting the killed nematode (Tunlid and Ahrén 2010). Clearly, although these two genes were from the same family of subtilisins, their expression profiles would suggest they have very different functions.

Upon infection, *C. elegans* responds by the activation of several intracellular signalling pathways which led to the expression of defensive gene products or effector molecules (Millet and Ewbank 2004; Gravato-Nobre and Hodgkin 2005). There are several distinct signal transduction cascades including DAF2/DAF16, MAP kinase and the TGF-β pathway as well as several others none of which appear totally independent but form an integrated signalling network. However, analysis of the expression profiles of the worm showed no distinct shifts which could be detected between one and four hours after infection (Fekete et al. 2008; Tunlid and Ahrén 2010). The most responsive up-regulated gene in this study was *dod-3* which is regulated by the transcription factor DAF-16. Other genes that responded to *M. haptotylum* infection included *cnc-4* that codes for one of eleven caenacin peptides that are secreted in response to *Drechmeria coniospora* infection, and *lec-8* and *lec-10* that encode galactose binding proteins (Fekete et al. 2008) which are also up-regulated during bacterial infection (Mallo et al. 2002; Gravato-Nobre and Hodgkin 2005). Interestingly, C-type lectins which have also been implicated in the response of *C. elegans* to infection (O'Rourke et al. 2006) were all down-regulated.

23.4.2 Trichoderma

Trichoderma spp. are a group of free-living fungi that are common in the rhizosphere. They can colonise root-surfaces and even establish themselves in the root epidemis. Roots colonized by *Trichoderma* grow better and confer resistance to abiotic (Yedidia et al. 2001, 2003; Harman et al. 2004) and biotic stresses (Herrera-Estrella and Chet 1998; Harman 2006). Various mechanisms have been proposed for their biological control activity against root pathogens which include both direct interactions such as parasitism, antibiosis and competition and indirect mechanisms such as plant systemic induced resistance (Harman et al. 2004, 2006; Viterbo et al. 2007). It is thought that in situations where biological control occurs it is a result of a multi-mechanism action (Sharon et al. 2010). *Trichoderma* spp. have been tested for their ability to control plant- parasitic nematodes (Windham et al. 1989; Reddy et al. 1996; Khan and Saxena 1997; Rao et al. 1998; Meyer et al. 2000, 2001; Sharon et al. 2001; Spiegel et al. 2007) but with mixed success. Clearly, if multiple modes of action are involved, understanding the importance of each of these, from attachment and infection processes through to antibiosis, will help in developing control agents with improved potential.

23.4.2.1 Conidial Attachment

Conidial attachment to the nematode surface is a key step in the infection process and appears to show a degree of specificity as conidia will attach to gelatinous egg matrix (gm) and nematodes that had had contact with the gm but not with gm-free J2s and eggs (Sharon et al. 2007). Application of antibodies to the surface coat of in-

fective juveniles increased attachment of conidia to the nematodes. This increase in parasitism possibly occurs through the process of bilateral binding in which the antibody forms a bridge between the conidium and the juvenile and thereby increases the rates of infection (Sharon et al. 2010). Further experiments have suggested that a Ca^{2+}-dependent lectin-carbohydrate interaction may be involved (Sharon et al. 2007, 2009).

23.4.2.2 Lytic Enzymes

Trichoderma spp. produce a whole array of different enzymes that are involved in plant defense and in biological control processes (Viterbo et al. 2002a, b; Markovich and Kononova 2003; Steyaert et al. 2003). Most research has focused on enzymes that degrade carbohydrates such as chitin, and the chitinolytic system of *Trichoderma* has been studied in detail (Kubicek et al. 2001). Chitinases are a family of enzymes, including exochitinases and endochitinases, that degrade chitin (Sharon et al. 2010). Nutrient starvation of the fungus and the products of chitin breakdown induce the chitinolytic enzyme expression system, whereas glucose and other simple sugars tend to suppress chitinolytic expression (Viterbo et al. 2002a). Although proteolytic enzymes have been less studied, a serine protease (Prb-1) produced by *T. atroviride* strain IMI 206040 inserted as multiple copies exhibited biological control potential against *R. solani* and also exhibited improved control potential against root-knot nematodes (Sharon et al. 2007). It is perhaps not surprising that fungal egg parasites that need to disrupt the eggshell probably require a combination of both chitinolytic and proteolytic enzymes (Morton et al. 2004).

23.4.2.3 Antibiotics

Trichoderma species can produce a variety of antibiotic compounds, which may contribute to the biological control processes. The nature and roles of antibiotic peptides that belong to the peptaibol group have been intensively studied (Szekeres et al. 2005). Peptaibols generally exhibit antimicrobial activity, which is thought to arise from their ability to form pores in lipid membranes. A peptaibol synthetase gene has been cloned (Wiest et al. 2002) and further studies suggested that peptaibols are critical in the chemical communication between *Trichoderma* and plants as triggers of non-cultivar-specific defence responses (Viterbo et al. 2007). *Trichoderma virens* produces gliotoxin and gliovirin and also peptaibols (Howell 2003). The antifungal action of enzymes reinforced by synergism with antibiotics was comprehensively reviewed by Kubicek et al. (2001) and this again points to multiple modes of action combining to generate functionality. Nematicidal activity against *M. javanica* J2s was detected in *T. atroviride* culture filtrates (CFs) and the active component/s had low molecular weight (MW) and heat sensitivity. Immature eggs, exposed to CFs exhibited reduced hatching rates, whereas hatching of mature eggs was enhanced. The effect of CF on eggs was caused by both the enzymatic fraction, which contained

proteases and chitinases, and by the low molecular mass component/s. (Sharon, Chet and Spiegel unpublished). Appropriate candidates responsible for such nematicidal activity might be antibiotic peptides, such as peptaibols (Sharon et al. 2007).

23.4.2.4 Fungal Genomics

Utilizing genomic and metabolomic means to understand *Trichoderma*-plant disease interactions (Shoresh and Harman 2008; Woo et al. 2009) can further enhance the efficacy of *Trichoderma* as a biocontrol agent against nematode pests. Comparative genomics and maximum likelihood based methods showed that three different chitinase subgroups have expanded in copy number in *Trichoderma* species, indicating an important role of these chitinases during the mycoparasitism process (Ihrmark et al. 2010). Several regions and amino acids have been identified in four chitinase genes from different *Trichoderma* species, that are likely to determine functional properties playing an important role in the interaction between plant/pathogens and mycoparasites, such as substrate-specificity, processivity or pH-optima. These approaches can help lead to a better understanding of multitrophic nematode/plant/*Trichoderma* interactions and improvement of the biocontrol activity.

23.4.3 Pasteuria penetrans Genomics Applied to Culturing and Host Specificity

Pasteuria penetrans is an endospore-forming Gram positive bacterium that is part of a group of invertebrate parasites that infect nematodes and water fleas (*Daphnia* spp). One of the members of this group, *P. ramosa*, which is a parasite of *Daphnia*, has become a model for studying host-parasite co-evolution (Decaestecker et al. 2007) and innate immunity (Little et al. 2003; Kurtz 2005). Within the plant-parasitic nematode context, research over the last twenty years has focused on *P. penetrans* and its potential to be developed into a biological control agent of plant-parasitic nematode pests (Davies 2009). The *Pasteuria* group is phylogenetically closely related to *Bacillus* and *Clostridium* spp. (Charles et al. 2005) and there are a number of different species, the designations of which are determined by descriptions of morphology, host-range, life-cycle and, more recently, 16s rDNA sequence (Table 23.1). The two main features of this bacterium that have prohibited its commercial development are its host specificity and the inability to mass produce it *in vitro.*

23.4.3.1 Genomic insights for *In Vitro* Culturing

Mass production of *P. penetrans* has had to rely on *in vivo* culturing methods of which the majority are adaptations of the method developed by Stirling and Wachtel

Table 23.1 Species of *Pasteuria* so far described and their invertebrate host

Species designation	Host	Reference
P. penetrans	*Meloidogyne* spp.	Sayre and Starr (1985)
P. thornei	*Pratylenchus brachyurus*	Starr and Sayre (1988)
P. nishizawae	*Heterodera and Globodera* spp.	Sayre et al. (1991)
P. ramosa	*Daphnia* spp.	Ebert et al. (1996)
P. usage	*Belonolaimus longicaudatus*	Giblin-Davis et al. (2003)
P. hartismeri	*M. ardenensis*	Bishop et al. (2007)

(1980). Briefly, infective root-knot nematode juveniles are exposed to endospores so that each juvenile is encumbered with 5–10 endospores. These encumbered second-stage juveniles are then placed around the roots of a tomato plant at 25°C. After 6–8 weeks the tomato roots containing nematodes infected with spores are washed free of soil and air dried. The roots are then milled and can be used as inoculum for application to soil. Such milled tomato root can contain as many as 1.3×10^9 endospores per gram of root powder (Pembroke and Gowen, personal communication). Early attempts to grow *Pasteuria in vitro* produced very limited success. However, more recently, Pasteuria Bioscience LLC, Florida, has developed a system for *in vitro* mass culture of *Pasteuria* (Hewlett et al. 2004). Their system can mass produce different species of *Pasteuria* and *Pasteuria* usage has been cleared by the Environmental Protection Agency (2009) for the control of sting nematode (*Belonolaimus longicaudatus*) on golf courses in the United States.

One of the problems encountered in culturing *Pasteuria in vitro* has been getting vegetative colonies to sporulate and comparative genomics has helped to shed light onto this problem. Sporulation in *Bacillus subtilis* has been extensively studied and is dependent on a phosphorelay pathway (Burbulys et al. 1991) in which a phosphoryl group is transferred to the regulator Spo0F through a group of five kinases that are under environmental regulation. Like all known regulators of this type, Spo0F requires a divalent metal ion to be present in the conserved aspartic acid pocket in order for phosphorylation to occur (Grimshaw et al. 1998) and Mg^{2+} has been shown to be important for this process (Zapf et al. 1996). More recently it has been suggested that metal cations may play a role in the structure and function of Spo0F and its involvement in the initiation of sporulation (Mukhopadhyay et al. 2004). Investigations of the effects of the divalent cations Ca^{2+}, Cu^{2+}, Mg^{2+}, and Mn^{2+} on the structure and function of *B. subtilis* Spo0F showed that they bound to the aspartic acid pocket and that while Mg^{2+} supports phosphotransfer from the kinase KinA to Spo0F the copper cation Cu^{2+} inhibited their phosphotransfer (Kojetin et al. 2005).

Interrogation of the *Pasteuria* survey sequence revealed genes with a high degree of similarity to genes involved in sporulation (Bird et al. 2003) and this included Spo0F. Alignment of Spo0F between *B. subtilis, B. anthracis and B. thuringiensis* and *P. penetrans* showed that key amino acids are conserved across these species. From the results discussed above it was hypothesised that the presence of Cu^{2+}, at non-lethal concentrations in the sporulation media for *B. subtilis* and the related bacterium *P. penetrans*, might inhibit endospore formation while continuing to permit vegetative growth. Indeed, subsequent experiments revealed that the absence

of Cu^{2+} in the media resulted in an increased number of sporulating cells (Kojetin et al. 2005). This result suggests that the availability of Cu^{2+} could be used to induce vegetative cells to enter sporulation.

23.4.3.2 Host Specificity and Endospore Attachment

The first stage of infection of a nematode by *Pasteuria* is when an endospore adheres to the surface of the nematode cuticle, and as discussed above, the Red Queen Hypothesis (Van Valen 1973, 1976) would suggest that within the context of an arms race it might be expected that functional diversity would co-evolve. Indeed, one isolate of *P. penetrans* does not adhere equally well to all populations of root-knot nematodes and the process of initial attachment is not linked to the phylogenetic diversity of the nematode populations tested (Davies et al. 2001). Five different monoclonal antibodies (Mabs) were raised to *Pasteuria* strain PP1 endospores from a single host female. Studies using these Mabs showed that there was a diversity of surface types as different sub-populations of the endospores of strain PP1 were recognised by each of the five different Mabs (Davies et al. 1994). Baiting the PP1 population of endospores with different species and races of root-knot nematode and using the Mabs to characterise the endospores that were adhering to each of the different populations of nematode, showed that different sub-populations of the endospores were adhering to the different nematode populations. This indicates that immunological heterogeneity in the surface of the endospore was related to heterogeneity present in the outer surface coat of the different nematode populations (Davies et al. 1994). Similar differences were also observed in the recognition of the surface of endospores between isolates of *Pasteuria* from different geographical regions by the different Mabs. One particular Mab, PP1/117, appeared to recognise the concave surface of the endospore to a greater extent than the upper surface, revealing that the density of the antigen was greater on the concave surface; pretreatment of these endospores with sugars or glycolytic enzymes reduced the ability of the Mab to bind, suggesting the Mab was recognising a carbohydrate epitope (Davies and Redden 1997).

The biochemical mechanism by which endospores of *Pasteuria* adhere to the nematode cuticle is poorly understood; however, it has been proposed that carbohydrate-lectin type interactions may be involved (Davies and Danks 1993). In a genomic survey sequence of *Pasteuria*, collagen-like genes were identified (Davies and Opperman 2006) which were initially thought to be contaminating genes from the nematode itself. However, subsequent analysis showed them to be similar to collagen-like genes identified in other closely related *Bacillus* species (Sylvestre et al. 2002, 2003, 2005) and similar genes, that appear to be highly polymorphic, have also been found in *P. ramosa* (Mouton et al. 2009). It has been suggested that these genes form fibres on the surface of the endospore and are important in attachment to the nematode cuticle (Davies 2009). If collagen-like fibres on the surface of the endospore are involved in attachment, what are the molecules involved on the surface of the nematode? Mucins are a family of polypeptides associated with both

the innate and adapted immune systems (Strous and Dekker 1992), which possess a polypeptide backbone that is highly glycosylated with sugar side chains. Glycosylation is predominantly *O*-linked through *N*-acetylgalactosamine (GalNAc) to serine and threonine residues within a variable number of tandem repeats (VNTR) regions of the polypeptide core (Hicks et al. 2000; Theodoropoulos et al. 2001). Although there is no information on the role of mucins in plant-parasitic nematodes, it is interesting that the genes *muc-2, muc-3* and *muc-4*, that are members of the TES-120 family of proteins present in *Toxocara canis* (Tetteh et al. 1999) and are responsible for surface coat variation and have homologues in *C. elegans*, are also all present in various species of plant-parasitic nematodes (Davies 2009).

The investigations outlined above have been brought together in a hypothesis which suggests that collagen-like proteins on the surface of the endospore are interacting with mucin-like molecules present on the surface coat of the infective juvenile in what has been described as a 'Velcro'-like mechanism (Davies 2009). Although the details of this system are yet to be elucidated, it is clear that both collagen-like molecules in respect to the endospore and mucin-like molecules with respect to the surface coat of the nematode would perfectly fit within the context of Van Valen's (1976) Red Queen Hypothesis.

23.5 Future Developments

Over the last two decades since the publication of Stirling's (1991) book on biological control, biology has undergone a revolution that combines the technologies of inexpensive and rapid DNA sequencing together with '*in silico*' computing developments in the growing field of genomics. The purpose of this chapter has been to try and clarify some of the ecological constraints that have prohibited the development of biological control from being a robust strategy to control nematode pests, by exploring some of the molecular and biochemical mechanisms that underpin host-parasite interactions. These areas can be brought together in the new and rapidly developing field of *ecological genomics*, which is multidisciplinary and integrates a number of disciplines (Fig. 23.1). By taking such an approach genomics can act as a catalyst for enabling ecology to become an increasingly hypothesis-driven area of research, and bring unity to these formally disparate areas of study. Whereas formerly gene function was analysed through knocking out genes, and was a fairly blunt tool, comparative genomics can look at natural genetic variation across ecoclines and examine how this plays a role in the functional ecology of host-parasite interactions. Therefore, from following a simple reductionist approach that runs from the gene, up through proteins and biochemical pathways to cells, tissues and organisms, a more integrated, holistic approach can be undertaken in which downward causation from communities of populations selected by the environment, lead to the structuring of particular genotypes engaged in multitrophic interactions between plant-hosts, nematode-pests and their parasites (Davies and Spiegel 2010).

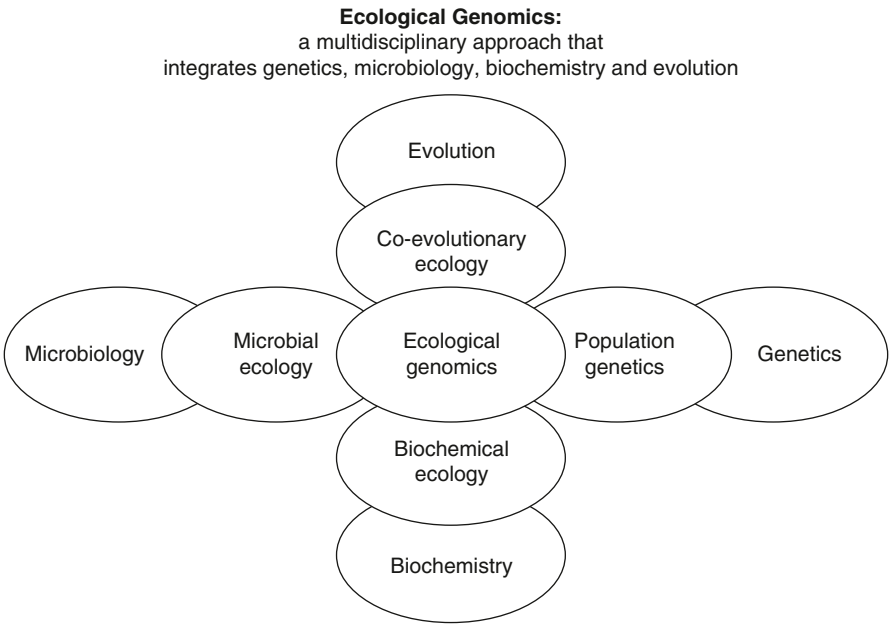

Fig. 23.1 Disciplines that need to be integrated within the context of ecological genomics as a strategy for the development of an understanding of biological control of nematodes. (Adapted from Straalen and Roelofs 2006)

23.5.1 Synthetic Biology

Very recently, the first microorganism was grown in which all the metabolic processes were controlled from an artificially constructed genome (Gibson et al. 2010). This arguably heralds the beginning of the epoch of synthetic biology, but the vision of designer organisms was first articulated in the 1970s when it was stated (Szybalski 1974):

> But the real challenge will start when we enter the synthetic biology phase of research in our field. We will then devise new control elements and add these new modules to the existing genomes or build up wholly new genomes.

This naturally begs the question as to whether the development of designer biological control agents is a future possibility. To date, the isolation, mass production and re-introduction of potential biological control agents has only met with limited success, but the idea of construction *de novo* of microorganisms with exactly the functionality required offers a multitude of possibilities. Although such approaches are in the medium to long term future, the development of this type of technology will not be beyond criticism, and many of the societal issues that confront the development of genetically modified crops will also need to be addressed regarding designer biological control agents (Davies and Spiegel 2010).

23.5.2 Commercial Development and Future Directions

In the relatively short term biological control will continue to offer products that will be of value to niche markets (Whipps and Davies 2000; Gowen et al. 2007; Hallman et al. 2009). However, the commercial market for the control of plant-parasitic nematodes in arable crops is immense and with the continued withdrawal of nematicides from the market due to legislation, the challenge to produce alternatives will remain. The continued study of biological control is important, not only for developing robust agents that can be applied and be expected to work with confidence, but also in the long term, in the epoch of synthetic biology, new combinations of functional traits can be assembled in novel systems that so far are unimaginable.

Acknowledgements KGD would like to acknowledge the support of the Biotechnological and Biological Sciences Research Council of the United Kingdom.

References

Abad P, Gouzy J, Aury JM, Castagnone-Sereno P, Danchin EGJ, Deleury E, Perfus-Barbeoch L, Anthouard V, Artiguenave F, Blok VC, Caillaud MC, Coutinho PM, Dasilva C, De Luca F, Deau F, Esquibet M, Flutre T, Goldstone JV, Hamamouch N, Hewezi T, Jaillon O, Jubin C, Leonetti P, Magliano M, Maier TR, Markov GV, McVeigh P, Pesole G, Poulain J, Robinson-Rechavi M, Sallet E, Segurens B, Steinbach D, Tytgat T, Ugarte E, van Ghelder C, Veronico P, Baum TJ, Blaxter M, Bleve-Zacheo T, Davis EL, Ewbank JJ, Favery B, Grenier E, Henrissat B, Jones JT, Laudet V, Maule AG, Quesneville H, Rosso MN, Schiex T, Smant G, Weissenbach J, Wincker P (2008) Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita.* Nature Biotechnology 26:909–915

Avendano F, Pierce FJ, Melakeberhan H (2004) The relationship between soybean cyst nematode seasonal population dynamics and soil texture. Nematology 6:511–525

Balogh J, Tunlid A, Rosen S (2003) Deletion of a lectin gene does not affect the phenotype of the nematode-trapping fungus *Arthrobotrys oligospora.* Fungal Genet Biol 39:128–135

Bardgett RD, Cook R, Yeates GW et al (1999) The influence of nematodes on below-ground processes in grassland ecosystems. Plant Soil 212:23–33

Bardgett RD, Hobbs PJ, Frostegard A (1996) Changes in soil: fungal biomass rations following reductions in the intensity of management of an upland grassland. Biol Fertil Soils 22:261–264

Barron GL (1977) The Nematode Destroying Fungi. Canadian Publications, Ontario, Canada

Bekku Y, Kimura M, Koizumi H (1997) Carbon input from plant to soil through root exudation in *Digitaria adscendens* and *Ambrosia artemisiifolia.* Ecol Res 12:305–312

Bird DM, Opperman CH, Davies KG (2003) Interactions between bacteria and plant parasitic nematodes: now and then. Int J Parasitol 33:1269–1276

Bishop AH, Gowen SR, Pembroke B, Trotter JR (2007) Morphological and molecular characteristics of a new species of *Pasteuria* parasitic on *Meloidogyne ardenensis.* J Invert Pathol 96:28–33

Blaxter M, Mann J, Chapman T, Thomas F, Whitton C, Floyd R, Abebe E (2005) Defining operational taxonomic units using DNA barcode data. Philos Trans R Soc London, Ser B: Biol Sci 360:1935–1943

Blaxter ML, De Ley P, Garey JR Liu LX, Scheldeman P, Vierstraete A, Vanfleteren JR, Mackey LY, Dorris M, Frisse LM, Vida JT, Thomas WK (1998) A molecular evolutionary framework for the phylum Nematoda. Nature 392:71–75

Borrebaeck CA, Mattiasson B, Nordbring-Hertz B (1984) Isolation and partial characterization of a carbohydrate-binding protein from a nematode-trapping fungus. J Bacteriol 159:53–56
Brinkman EP, Duyts H, van der Putten WH (2005) Consequences of variation in species diversity in a community of root-feeding herbivores for nematode dynamics and host plant biomass. Oikos 110:417–427
Burbulys D, Trach KA, Hoch JA (1991) Initiation of sporulation in *B. subtilis* is controlled by a multicomponent phosphorelay. Cell 64:545–552
Charles L, Carbonne I, Davies KG, Bird D, Burke M, Kerry BR, Opperman CH (2005) Phylogenetic analysis of *Pasteuria penetrans* using multiple genetic loci. J Bacteriol 187:5700–5708
Clark MF (1994) Immunodiagnosis methods using polyclonal and monoclonal antibodies. In: Hawksworth DL (ed) The identification and characterisation of pest organisms. CAB International, Wallingford, pp 377–393
Cobb NA (1920) Transferance of nematodes (Mononchs) from place to place for economic purposes. Science 51:640–641
Cook R, Starr, JL (2006) Resistant Cultivars. In: Perry RN, Moens M (eds) Plant nematology CABI International, Wallingford, pp 370–391
Costa SR, Kerry BR, Bardgett R, Davies KG (2006) Exploitation of immunofluorescence for the quantification and characterisation of small numbers of *Pasteuria* endospores. FEMS Microbiol Ecol 58:593–600
Costa SR, van der Putten WH, Kerry BR (2010) Microbial ecology and nematode control in natural ecosystems. In: Davies KG, Spiegel Y (eds) Biological control of plant parasitic nematodes: building coherence between microbial ecology and molecular mechanisms. Springer, Berlin, Dortrecht
Darby C (2005) Interactions with microbial pathogens. WormBook, pp 1–15
Davies KG (1994) A nematode case study focusing on the application of serology. In: Hawksworth DL (ed) The identification and characterisation of pest organisms. CAB International, Wallingford, pp 395–413
Davies KG (2009) Understanding the interaction between an obligate hyperparasitic bacterium, *Pasteuria penetrans* and its obligate plant parasitic nematode host, *Meloidogyne* spp. Adv Parasitol 68:211–245
Davies KG, Carter B (1995) Comparison of immunoassays for the quantification of root-knot nematodes extracted from soil. EPPO Bull 25:367–375
Davies KG, Danks C (1993) Carbohydrate/protein interactions between the cuticle of infective juveniles of *Meloidogyne incognita* and spores of the obligate hyperparasite *Pasteuria penetrans.* Nematologica 39:54–64
Davies KG, Lander EB (1992) Immunological differentiation of root-knot nematodes (*Meloidogyne* spp.) using monoclonal and polyclonal antibodies. Nematologica 38:353–366
Davies KG, Opperman CH (2006) A potential role for collagen in the attachment of *Pasteuria penetrans* to nematode cuticle. IOBC/wprs Bull 29:11–15
Davies KG, Redden M (1997) Diversity and partial characterisation of putative virulence determinants in *Pasteuria penetrans*, the hyperparasite of root-knot nematodes. J Appl Microbiol 83:227–235
Davies KG, Spiegel Y (2010) Root patho-systems nematology and biological control. In: Davies KG, Spiegel Y (eds) Biological control of plant- parasitic nematodes: building coherence between microbial ecology and molecular mechanisms. Springer, Berlin, Dortrecht
Davies KG, Redden M, Pearson TK (1994) Endospore heterogeneity in *Pasteuria penetrans* related to attachment to plant-parasitic nematodes. Lett Appl Microbiol 19:370–373
Davies KG, Curtis RH, Evans K (1996) Serologically based diagnostic and quantification tests for nematodes. Pestic Sci 47:81–87
Davies KG, Fargette M, Balla G, Daudi A, Duponnois R, Gowen SR, Mateille T, Phillips MS, Sawadogo S, Trivino C, Vouyoukalou E, Trudgill DL (2001) Cuticle heterogeneity as exhibited by *Pasteuria* spore attachment is not linked to the phylogeny of parthenogenetic root-knot nematodes (*Meloidogyne* spp.) Parasitology 122:111–120

Decaestecker E, Gaba S, Raeymaekers JA, Stoks R, Van Kerckhoven L, Ebert D, De Meester L (2007) Host-parasite 'Red Queen' dynamics archived in pond sediment. Nature 450:870–873

de la Pena E, Vandegehuchte M, Bonte D et al (2008) Analysis of the specificity of three root-feeders towards grasses in coastal dunes. Plant Soil 310:113–120

De Mesel I, Derycke S, Moens T, van der Gucht K, Vincx M, Swings J (2004) Top-down impact of bacterivorous nematodes on the bacterial community structure: a microcosm study. Environ Microbiol 6:733–744

Denton CS, Bardgett RD, Cook R, Hobbs PJ (1999) Low amounts of root herbivory positively influence the rhizosphere microbial community in a temperate grassland soil. Soil Biol Biochem 31:155–165

Dijksterhuis J, Veenhuis M, Harder W, Nordbring-Hertz B (1994) Nematophagous fungi: physiological aspects and structure-function relationships. Adv Microb Physiol 36:111–143

Ebert D amd Hamilton WD (1996) Sex against virulence: the coevolution of parasitic diseases. Trends Ecol Evol 11:A79–A82

Ebert D, Rainey P, Embley TM, Scholtz D (1996) Development, life cycle, ultrastructure and phylogenetic position of *Pasteuria ramosa* Metchnikoff 1888: rediscovery of an obligate endoparasite of *Daphnia magna* Straus. Philos Trans R Soc Lond B 351:1689–1701

Environmental Protection Agency of the United States (2009) Biopesticide registration action document *Pasteuria usage* PC code 006545. http://www.epa.gov/opp00001/biopesticides/ingredients/tech_docs/brad_006545.pdf

Ettema CH, Wardle DA (2002) Spatial soil ecology. Trends Ecol Evol 17:177–183

Fekete C, Tholander M, Rajashekar B, Ahren D, Friman E, Johansson T, Tunlid A (2008) Paralysis of nematodes: shifts in the transcriptome of the nematode-trapping fungus *Monacrosporium haptotylum* during infection of *Caenorhabditis elegans*. Environ Microbiol 10:364–375

Floyd R, Abebe E, Papert A, Blaxter M (2002) Molecular barcodes for soil nematode identification. Mol Ecol 11:839–850

Foucher ALJL, Bongers T, Noble LR, Wilson JM (2004) Assessment of nematode biodiversity using DGGE of 18S rDNA following extraction of nematodes from soil. Soil Biol Biochem 36:2027–2032

Fraser C, Alm EJ, Polz MF, Spratt BG, Hanage WP (2009) The bacterial species challenge: making sense of genetic and ecological diversity. Science 323:741–746

Fuchs BB, Mylonakis E (2006) Using non-mammalian hosts to study fungal virulence and host defense. Curr Opin Microbiol 9:346–351

Gair R, Mathias PL, Harvey PN (1969) Studies of cereal nematode populations and cereal yields under continuous or intensive culture. Ann Appl Biol 63:503–512

Giblin-Davis RMD, Williams S, Bekal S, Dickson DW, Brito JA, Becker JO, Preston JF (2003) '*Candidatus* Pasteuria usgae' sp. nov., an obligate endoparasite of the phytoparasitic nematode, *Belonolaimus longicaudatus*. Int J Syst Evol Microbiol 53:197–200

Gibson DG, Glass JI, Lartigue C, Noskov VN, Chuang RY, Algire MA, Benders GA, Montague MG, Ma L, Moodie MM, Merryman C, Vashee S, Krishnakumar R, Assad-Garcia N, Andrews-Pfannkoch C, Denisova EA, Young L, Qi ZQ, Segall-Shapiro TH, Calvey CH, Parmar PP, Hutchison CA, Smith HO, Venter JC (2010) Creation of a bacterial cell controlled by a chemically synthesized genome. Science 329:52–56

Gowen SR, Davies KG, Pembroke B (2007) Potential use of *Pasteuria* spp. in the management of plant parasitic nematodes. In: Ciancio A, Mukerji KG (eds) Integrated management and biocontrol of vegetable and grain crops nematodes. Springer, Dortrecht, pp 197–210

Gravato-Nobre MJ, Hodgkin J (2005) *Caenorhabditis elegans* as a model for innate immunity to pathogens. Cell Microbiol 7:741–751

Grimshaw CE, Huang S, Hanstein CG, Strauch MA, Burbulys D, Wang L, Hoch JA, Whiteley JM (1998) Synergistic kinetic interactions between components of the phosphorelay controlling sporulation in *Bacillus subtilis*. Biochemistry 37:1365–1375

Hallmann J, Davies KG, Sikora R (2009) Biological control using microbial pathogens, endophytes and antagonists. In: Perry RN, Moens M, Starr JL (eds) Root-knot nematodes. CABI International, Wallingford

Hallmann J, Sikora RA (2010) Endophytic fungi. In: Davies KG, Spiegel Y (eds) Biological control of plant- parasitic nematodes: building coherence between microbial ecology and molecular mechanisms. Springer, Berlin, Dortrecht

Harman GE (2006) Overview of mechanisms and uses of *Trichoderma* spp. Phytopathology 96:190–194

Harman GE, Howell CR, Viterbo A, Chet I, Lorito M (2004) *Trichoderma* spp.—opportunistic avirulent plant symbionts. Nature Microbiol Rev 2:43–56

Herrera-Estrella A, Chet I (1998) Biocontrol of bacteria and phytopathogenic fungi. In: Altman A (ed) Agricultural biotechnology. Marcel Dekker, New York, Basel

Hewlett TE, Gerber JF, Smith KS (2004) *In vitro* culture of *Pasteuria penetrans*. In: Cook RC, Hunt DJ (eds) Nematology monographs and perspectives, vol 2. Proceedings of the fourth international congress of nematology, pp 175–185

Hicks SJ, Theodoropoulos G, Carrington SD, Corfild AP (2000) The role of mucins in host-parasite interactions. Part I: protozoan parasites. Parasitol Today 16:476–481

Hirsch P, Atkins SD, Mauchline TH, Morton CO, Davies KG, Kerry BR (2001) Techniques for studying nematophagous fungi in the root environment. Plant Soil 232:21–30

Hodge A (2006) Plastic plants and patchy soils. J Exp Botany 57:401–411

Hodgkin J, Partridge FA (2008) *Caenorhabditis elegans* meets microsporidia: the nematode killers from Paris. PLoS Biol 6:2634–2637

Holterman M, van der Wurff A, van den Elsen S, van Megen H, Bongers T, Holovachov O, Bakker J, Helder J (2006) Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. Mol Biol Evol 23:1792–1800

Holterman M, Karssenm G, van den Elsen S, van Megen H, Bakker J, Helder J (2009) Small subunit rDNA-based phylogeny of the Tylenchida sheds light on relationships among some high-impact plant-parasitic nematodes and the evolution of plant feeding. Phytopathology 99:227–235

Howell CR (2003) Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. Plant Dis 87:4–10

Ihrmark K, Asmail N, Ubhayasekera W, Melin P, Stenlid J, Karlsson M (2010) Comparative molecular evolution of *Trichoderma* chitinases in response to mycoparasitic interaction. Evol Bioin 6:1–26

Jones FGW, Jones MG (1974) Pests of field crops. Edward Arnold Ltd., London

Kawecki TJ (1998) Red queen meets Santa Rosalia: arms races and the evolution of host specialization in organisms with parasitic lifestyles. Am Nat 152:635–651

Kerry BR (1975) Fungi and the decrease of cereal cyst-nematode populations in cereal monoculture. EPPO Bull 5:353–361

Kerry BR, Crump DH (1977) Observations on fungal parasites of females and eggs of the cereal cyst-nematode, *Heterodera avenae*, and other cyst nematodes. Nematologica 23:193–201

Kerry BR, Crump DH, Mullen LA (1982) Studies of the cereal cyst nematode *Heterodera avenae* under continuous cereals, 1975–1978. II Fungal parasitism of nematode eggs and females. Ann App Biol 100:489–499

Kerry BR, Hirsch PR (2010) Ecology of *Pochonia chlamydosporia* in the rhizosphere at the population, whole organism and molecular scales. In: Davies KG, Spiegel Y (eds) Biological control of plant- parasitic nematodes: building coherence between microbial ecology and molecular mechanisms Springer, Berlin, Dortrecht

Khan TA, Saxena SK (1997) Effect of root-dip treatment with fungal filtrates on root penetration, development and reproduction of *Meloidogyne javanica* on tomato. Int J Nematol 7:85–88

Kojetin DJ, Thompson RJ, Benson LM, Naylor S, Waterman J, Davies KG, Opperman CH, Stephenson K, Hoch JA, Cavanagh J (2005) Structural analysis of divalent metals binding to the *Bacillus subtilis* response regulator Spo0F: the possibility for *in vitro* metalloregulation in the initiation of sporulation. Biometals 18:449–466

Kubicek CP, Mach RL, Peterbauer CK, Lorito M (2001) *Trichoderma*: from genes to biocontrol. J Plant Pathol 83:11–23

Kurtz J (2005) Specific memory within innate immune systems. Trends Immunol 26:186–192

Linford MB (1937) Stimulated acticity of natural enemies of nematodes. Science 85:123–124
Linford MB, Yap F, Oliveira JM (1938) Reduction of soil populations of the root-knot nematode during decomposition of organic matter. Soil Sci 45:127–141
Little TJ, O'Connor B, Colegrave NM, Watt K, Read AF (2003) Maternal transfer of strain specific immunity in an invertebrate Curr Biol 13:489–491
Mallo GV, Kurz CL, Couillault C, Pujol N, Granjeaud S, Kohara Y, Ewbank JJ (2002) Inducible antibacterial defense system in *C. elegans.* Curr Biol 12:1209–1214
Markovich NA, Kononova GL (2003) Lytic enzymes of *Trichoderma* and their role in plant defense from fungal diseases: a review. Appl Biochem Microbiol 39:389–400
McSorley R, Duncan LW (2004) Population dynamics. Nematology: advances and perspective, vol 1: nematode morphology, physiology and ecology. CABI International, Wallingford, pp 469–492
McSorley R, Frederick JJ (2004) Effect of extraction method on perceived composition of the soil nematode community. Appl Soil Ecol 27:55–63
Mendoza De Gives PM, Davies KG, Clark SJ, Behnke JM (1999) Predatory behaviour of trapping fungi against srf mutants of Caenorhabditis elegans and different plant and animal parasitic nematodes. Parasitology 119:95–104
Meyer SLF, Massoud SI, Chitwood DJ, Roberts DP (2000) Evaluation of *Trichoderma virens* and *Burkholderia cepacia* for antagonistic activity against root-knot nematode, *Meloidogyne incognita.* Nematology 2:871–879
Meyer SLF, Roberts DP, Chitwood DJ, Carta LK, Lumsden RD, Mao WL (2001) Application of *Burkholderia cepacia* and *Trichoderma virens*, alone and in combinations, against *Meloidogyne incognita* on bell pepper. Nematropica 31:75–86
Millet AC, Ewbank JJ (2004) Immunity in Caenorhabditis elegans. Curr Opin Immunol 16:4–9
Mitra RM, Shaw SL, Long SR (2004) Six nonnodulating plant mutants defective for Nod factor-induced transcriptional changes associated with the legume-rhizobia symbiosis. Proc Natl Acad Sci U S A 101:10217–10222
Morton CO, Hirsch PR, Kerry B (2004) Infection of plant-parasitic nematodes by nematophagous fungi—a review of application of molecular biology to understand infection processes and to improve biological control. Nematology 6:161–170
Mouton L, Traunecker E, McElroy K, Du Pasquier L, Ebert D (2009) Identification of a polymorphic collagen-like protein in the crustacean bacteria *Pasteuria ramosa.* Res Microbiol 160:792–799
Mukhopadhyay D, Sen U, Zapf J, Varughese KI (2004) Metals in the sporulation phosphorelay: manganese binding by the response regulator Spo0F. Acta Crystallogr Sect D: Biol Crystallogr 60:638–645
Muyzer G, Dewaal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel-electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16s ribosomal-RNA. Appl Envon Microbiol 59:695–700
Nordbring-Hertz B, Chet I (1988) Fungal lectins and agglutinins. In: Mirelman D (ed) Microbial lectins and agglutinins: properties and biological activity. Wiley, New York, pp 393–408
Nordbring-Hertz B, Mattiasson B (1979) Action of a nematode-trapping fungus shows lectin-mediated host-microorganism interaction. Nature 281:477–479
O'Donnell AG (1994) Quantitative and qualitative analysis of fatty acids in the classification and identification of microorganisms. In: Hawksworth DL (ed) The Identification and characterisation of pest organisms. CAB International, Wallingford, pp 323–335
O'Donnell AG, O'Donnell AG Colvan SR, Malosso E, Supaphol S (2005) Twenty years of molecular analysis of bacterial communities and what have we learned about function? In: Bardgett RD, Usher MB, Hopkins DW (eds) Biological diversity and function in soils. Cambridge University Press, Cambridge, pp 44–56
Opperman CH, Bird DM, Williamson VM, Rokhsar DS, Burke M, Cohn J, Cromer J, Diener S, Gajan J, Graham S, Houfek TD, Liu Q, Mitros T, Schaff J, Schaffer R, Scholl E, Sosinski BR, Thomas VP, Windham E (2008) Sequence and genetic map of *Meloidogyne hapla*: a compact nematode genome for plant parasitism. Proc Natl Acad Sci U S A 105:14802–14807

O'Rourke D, Baban D, Demidova M, Mott R, Hodgkin J (2006) Genomic clusters, putative pathogen recognition molecules, and antimicrobial genes are induced by infection of *C. elegans* with *M. nematophilum.* Genome Res 16:1005–1016

Powers T (2004) Nematode molecular diagnostics: from bands to barcodes. Ann Rev Phytopathol 42:367–383

Pramer D, Kuyama S (1963) Symposium on biochemical bases of morphogenesis in fungi. II: nemin and the nematode-trapping fungi. Bacteriol Rev 27:282–292

Pramer D, Stoll NR (1959) Nemin: a morphogenetic substance causing trap formation by predaceous fungi. Science 129:966–967

Premachandran D, Pramer D (1984) Role of N-Acetylgalactosamine-specific protein in trapping of nematodes by *Arthrobotrys oligospora.* Appl Environ Microbiol 47:1358–1359

Rao MS, Reddy PP, Nagesh M (1998) Evaluation of plant based formulations of *Trichoderma harzianum* for the management of *Meloidogyne incognita* on egg plant. Nematol Medit 26:59–62

Reddy PP, Rao MS, Nagesh M (1996) Management of citrus nematode, *Tylenchulus semipenetrans*, by integration of *Trichoderma harzianum* with oil cakes. Nematol Medit 24:265–267

Reitz M, Rudolph K, Schroder I, Hoffmann-Hergarten S, Hallmann J, Sikora RA (2000) Lipopolysaccharides of *Rhizobium etli* strain G12 act in potato roots as an inducing agent of systemic resistance to the infection by the cyst nematode *Globodera pallida.* Appl Environm Microbiol 66:3515–3518

Robinson MP, Butcher G, Curtis RH, Davies KG, Evans K (1993) Characterisation of a 34kD protein from potato cyst nematodes, using monoclonal antibodies with potential for species diagnosis. Ann Appl Biol 123:337–347

Rosén S, Ek B, Rask L, Tunlid A (1992) Purification and characterization of a surface lectin from the nematode-trapping fungus *Arthrobotrys oligospora.* J Gen Microbiol 138:2663–2672

Saiki R, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, Arnheim N (1985) Enzymic amplification of β-globulin genomic sequences and restriction site analysis for the diagnosis of sickle cell anemia. Science 230:1350–1354

Sayre RM, Starr MP (1985) *Pasteuria penetrans* (*ex* Thorne, 1940) nom. rev., comb. n., sp. n., a mycelial and endospore-forming bacterium parasitic in plant-parasitic nematodes. Proc Helminthol Soc Wash 52:149–165

Sayre RM, Wergin WP, Schmidt JM, Starr MP (1991) *Pasteuria nishizawae* sp. nov., a mycelial and endosporeforming bacterium parasitic on cyst nematodes of genera *Heterodera* and *Globodera.* Res Microbiol 142:551–564

Sharon N, Lis H (2004) History of lectins: from hemagglutinins to biological recognition molecules. Glycobiol 14:53R–62R

Sharon E, Bar-Eyal M, Chet I (2001) Biocontrol of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum.* Phytopathology 91:687–669

Sharon E, Chet I, Viterbo A, Bar-Eyal M, Nagan H, Samuels GJ, Spiegel Y (2007) Parasitism of *Trichoderma* on *Meloidogyne javanica* and role of the gelatinous matrix. Eur J Plant Pathol 118:247–225

Sharon E, Chet I, Spiegel Y (2009) Improved attachment and parasitism of *Trichoderma* on *Meloidogyne javanica in vitro.* Eur J Plant Pathol 123:291–299

Sharon E, Chet I, Spiegel Y (2010) *Trichoderma* as a biocontrol agent. In: Davies KG, Spiegel Y (eds) Biological control of plant- parasitic nematodes: building coherence between microbial ecology and molecular mechanisms. Springer, Berlin, Dortrecht

Shoresh M, Harman G E (2008) The molecular basis of shoot responses of maize seedlings to *Trichoderma harzianum* T22 inoculation of the root: a proteomic approach. Plant Physiol 147:2147–2163

Spiegel Y, Sharon E, Bar-Eyal M (2007) Evaluation and mode of action of *Trichoderma* isolates as biocontrol agents against plant-parasitic nematodes. IOBC/WPRS Bull 30:129–133

Starr MP, Sayre RM (1988) *Pasteuria thornei* sp. nov. and *Pasteuria penetrans* sensu stricto emend., mycelial and endospore-forming bacteria parasitic, respectively, on plant-parasitic nematodes of the genera *Pratylenchus* and *Meloidogyne.* Ann Inst Pasteur Microbiol 139:11–31

Steyaert JM, Ridgway HJ, Elad Y, Stewart A (2003) Genetic basis of mycoparasitism: a mechanism of biological control by species of *Trichoderma.* N Zeal J Crop Hortic Sci 31:281–291
Stirling GR (1991) Biological control of plant parasitic nematodes: progress, problems and prospects. CAB International, Wallingford
Stirling GR, Wachtel MF (1980) Mass production of *Bacillus penetrans* for the biological control of root-knot nematodes. Nematologica 26:308–312
Straalen NM, Roelofs D (2006) An introduction to ecological genomics. Oxford University Press, Oxford
Strous GJ, Dekker J (1992) Mucin-type glycoproteins. Crit Rev Biochem Mol Biol 27:57–92
Sylvestre P, Couture-Tosi E, Mock M (2002) A collagen-like surface glycoprotein is a structural component of the *Bacillus anthracis* exosporium. Mol Microbiol 45:169–178
Sylvestre P, Couture-Tosi E, Mock M (2003) Polymorphism in the collagen-like region of the *Bacillus anthracis* BclA protein leads to variation in length in the exosporium filament length. J Bacteriol 185:5155–5163
Sylvestre P, Couture-Tosi E, Mock M (2005) Contribution of ExsFA and ExsFB proteins to the localisation of BclA on the spoe surface and to the stability of the *Bacillus anthracis* exosporium. J Bacteriol 187:5122–5128
Szekeres A, Leitgeb B, Kredics L, Antal, ZS, Hatvani, L, Manczinger, L, Vagvolgyi, CS (2005) Peptaibols and related peptaibiotics of *Trichoderma.* A review. Acta Microbiol Immunol Hung 52:137–168
Szybalski W (1974) *In vivo* and *in vitro* initiation of transcription, In: Kohn A, Shatkay A (Eds) Control of gene expression and discussion. Plenum Press, New York
Tetteh KKA, Loukas A, Tripp C, Maizels RM (1999) Identification of abundantly expressed novel and conserved genes from the infective larval stage of *Toxacara canis* by an expressed sequence tag strategy. Infect Immunol 67:4771–4779
Thorne G (1927) The life history, habits and economic importance of some mononchs. J Agric Res 34:265–286
Theodoropoulos G, Hicks SJ, Corfield AP, Miller BG, Carrington SD (2001) The role of mucins in host-parasite interactions: Part II: Helminth parasites. Trends Parasitol 17:130–135
Trudgill DL (1991) Resistance to and tolerance of plant parasitic nematodes in plants. Annu Rev Phytopathol 29:167–192
Tunlid A, Ahrén D (2010) Molecular mechanisms of the interaction between nematode-trapping fungi and nematodes—lessons from genomics. In: Davies KG, Spiegel Y (eds) Biological control of plant- parasitic nematodes: building coherence between microbial ecology and molecular mechanisms. Springer, Berlin, Dortrecht
Tunlid A, Åhman J, Oliver RP (1999) Transformation of the nematode-trapping fungus *Arthrobotrys oligospora.* FEMS Microbiol Lett 173:111–116
Van den Berg W, Rossing WAH (2005) Generalised linear dynamics of a plant-parasitic nematode population and the economic evaluation of crop rotations. J Nematol 37:55–65
Van der Putten WH, vet LEM, Harvey JA, Harvey JA, Wackers FL (2001) Linking above- and belowground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. Trends Ecol Evol 16:547–554
Van Elsas JD, Duarte GF, Keijzer-Wolters A, Smit E (2000) Analysis of the dynamics of fungal communites in soil via fungal-specific PCR of soil DNA followed by denaturing gradient gel electrophoresis. J Microbiol Methods 43:133–151
Van Valen L (1973) A new evolutionary law. Evol Theory 1:1–30
Van Valen L (1976) The red queen. Am Nat 110:809–810
Varley GC, Gradwell GR, Hassell MP (1973) Insect population ecology: an analytical approach. Blackwell, Oxford
Viterbo A, Montero M, Ramot O, Friesem D, Monte E, Llobell A, Chet I (2002a) Expression regulation of the endochitinase chit36 from *Trichoderma asperellum* (*T. harzianum* T-203). Curr Genet 42:114–122
Viterbo A, Ramot O, Chernin L, Chet I (2002b) Significance of lytic enzymes from *Trichoderma* spp. in the biocontrol of fungal plant pathogens. Antonie van Leeuwenhoek 81:549–556

Viterbo A, Inbar J, Hadar Y, Chet I (2007) Plant disease biocontrol and induced resistance via fungal mycoparasites. In: Kubicek CP, Deruzhinina IS (eds) The mycota IV: environmental and microbial relationships, 2nd edn. Springer, Berlin, Dortrecht

Waite IS, O'Donnell AG, Harrison A, Davies JT, Colvan SR, Ekschmitt K, Dogan H, Wolters V, Bongers T, Bongers M, Bakonyi G, Nagy P, Papatheodorou EM, Stamou GP, Bostrom S (2003) Design and evaluation of nematode 18S rDNA primers for PCR and denaturing gradient gel electrophoresis (DGGE) of soil community DNA. Soil Biol Biochem 35:1165–1173

Watt M, Silk WK, Passioura JB (2006) Rates of root and organism growth, soil conditions, and temporal and spatial development of the rhizosphere. Ann of Botany 97:839–855

Whipps JM, Davies KG (2000) Success in biological control of plant pathogens and nematodes by microorganisms. In: Gurr G, Wratten S (eds) Biological control: measures of success. Kluwer, Dordrecht

Wiest A, Grzegorski D, Xu B-W, Goulard C, Rebuffat S, Ebbole DJ, Bodo B, Kenerley C (2002) Identification of peptaibols from *Trichoderma virens* and cloning of a peptaibol synthetase. J Biol Chem 277:20862–20868

Windham GL, Windham MT, Williams WP (1989) Effects of *Trichoderma* spp. on maize growth and *Meloidogyne arenaria* reproduction. Plant Dis 73:493–494

Woo SL, Ruocco M, Vinale F, Lorito M (2009) The *Trichoderma*-plant-pathogen interaction: understanding the mechanisms and improving biocontrol. In: Elad Y, Maurhofer M, Keel C et al (eds) Biological control of fungal and bacterial plant pathogens. IOBC/wprs Bull 43:83–88

Wood FH (1973) Nematode feeding relationships. Feeding relationships of soil-dwelling nematodes. Soil Biol Biochem 5:528–537

Wu TH, Ayres E, Li G et al (2009) Molecular profiling of soil animal diversity in natural ecosystems: incongruence of molecular and morphological results. Soil Biol Biochem 41:849–857

Wurst S, van Beersum S, Wagenaar R, Bakx-Schotman T, Drigo B, Janzik I, Lanoue A, Van der Putten WH (2009) Plant defence against nematodes is not mediated by changes in the soil microbial community. Funct Ecol 23:488–495

Yeates GW, Bongers T, de Goede RGM, Freckman DW, Georgieva SS (1993) Feeding habits in soil nematode families and genera—an outline for soil ecologists. J Nematol 25(3):315–331

Yedidia I, Srivastva AK, Kapulnik Y et al (2001) Effect of *Trichoderma* harzianum on microelement concentrations and increased growth of cucumber plants. Plant Soil 235:235–242

Yedidia I, Shoresh M, Kerem Z et al (2003) Concomitant induction of systemic resistance to *Pseudomonas syringae* pv. *lachrymans* in cucumber by *Trichoderma asperellum* (T-203) and accumulation of phytoalexins. Appl Environ Microbiol 69:7343–7353

Zapf JW, Hoch JA, Whiteley JM (1996) A phosphotransferase activity of the Bacillus subtilis sporulation protein Spo0F that employs phosphoramidate substrates. Biochemistry 35:2926–2933

Zheng SJ, Dicke M (2008) Ecological genomics of plant – insect interactions: from gene to community. Plant Physiol 146:812–817