Corky Root Disease Management in Organic Tomato Production

Composts, Fungivorous Nematodes and Grower Participation

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Abstract

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The role of composts and fungivorous nematodes in the control of corky root disease of tomato caused by the soil-borne fungus *Pyrenochaeta lycopersici* was investigated in organic production systems. The composts evaluated were a green manure compost prepared from red clover, a horse manure compost and two garden waste composts. Composts were mixed (20% v/v) with soil naturally infested with *P. lycopersici*. Three-week old tomato seedlings were transplanted in compost/soil mix for 10 weeks in the greenhouse to investigate potential suppressive effects of composts on corky root disease. The fungivorous nematodes studied were *Aphelenchus avenae* and *Aphelenchoides* spp. The suitability of *P. lycopersici* as a host for the fungivorous nematodes was determined on agar plates. The effects of the fungivorous nematodes on corky root disease were then investigated by inoculating fungivorous nematodes into *Pyrenochaeta*-infested soil in greenhouse trials. In addition, fungivorous nematodes were inoculated into the compost-amended infested soils to determine the combined effect of the composts and fungivorous nematodes on corky root disease. Other potential measures for controlling corky root disease, such as use of mulch, break crop, grafted tomato plants, composted *Pyrenochaeta*-infested soil and commercially available bio-control agents, were evaluated in participation with a group of commercial organic tomato growers.

A garden waste compost with low NH_4-N concentration and high Ca concentration reduced corky root disease. Populations of the fungivorous nematodes developed well on the culture of *P. lycopersici* in the *in vitro* tests. In greenhouse experiments, *A. avenae* reduced corky root disease severity but *Aphelenchoides* spp. did not. When *A. avenae* was applied in a commercial greenhouse soil, however, no disease reduction by this fungivorous nematode was observed. Furthermore, no disease reduction effects was observed with combined application of composts and fungivorous nematodes to *Pyrenochaeta*-infested soil.

Overall, no single treatment provided a sufficiently high degree of corky root disease control to be recommended to growers. The study emphasises the need for integration of different measures to keep corky root disease below an economically tolerable threshold level.

Keywords: *Aphelenchus avenae*, *Aphelenchoides* spp., biological control, garden waste compost, green manure compost, horse manure compost, participatory research, *Pyrenochaeta lycopersici*

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To my father *Abu Motalib* and
my mother *Tahmina Begum*

Contents

Appendix

Papers I-IV

The thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I. Hasna, M.K., Mårtensson, A., Persson, P. & Rämert, B. Use of composts to manage corky root disease in organic tomato production. *Annals of Applied Biology*. **In Press**.

II. Hasna, M.K., Insunza, V., Lagerlöf, J. & Rämert, B. 2007. Food attraction and population growth of fungivorous nematodes with different fungi. *Annals of Applied Biology 151*, 175-182.

III. Hasna, M.K., Lagerlöf, J. & Rämert, B. Effects of fungivorous nematodes on corky root disease of tomato grown in compost-amended soil. *Acta Agriculturae Scandinavica Section B, Soil and Plant Science*. **In Press**.

IV. Hasna, M.K., Ögren, E., Persson, P., Mårtensson, A. & Rämert, B. Management of corky root disease of tomato in participation with organic tomato growers (Manuscript).

The contribution of Mahbuba Kaniz Hasna to the papers included in this thesis was as follows:

I. Planned the experiments together with co-authors. Performed greenhouse experiments and laboratory work concerning analysis of microbial activity and microbial population of soil, composts and soil-compost mixtures. Carried out writing of the paper, guided by Mårtensson, Persson and Rämert.

II. Planned the experiments together with co-authors. Performed laboratory works in co-operation with Insunza. Carried out writing of the paper supported by Insunza, Lagerlöf and Rämert.

III. Planned the experiments together with co-authors. Performed greenhouse experiments and most of the analyses of nematode populations. Carried out writing of the paper guided by Lagerlöf and Rämert.

IV. Planned the experiments together with co-authors. Performed laboratory work, greenhouse experiments and trials in the greenhouse of an organic tomato grower in co-operation with co-authors. Carried out writing of the paper supported by Ögren, Persson, Mårtensson and Rämert.

Introduction

The soil-borne fungal disease corky root of tomato, caused by *Pyrenochaeta lycopersici* Schneider & Gerlach, is a disease of concern for many tomato-growing areas, both in greenhouses using soil as growing substrate and in the field. The disease has been identified as the most common and economically important disease in Swedish organic tomato production (Forsberg, Sahlström & Ögren, 1999). Occurrence of *P. lycopersici* has been reported in many parts of the world, for example in Germany (Gerlach & Schneider, 1964), England (Last, Ebben & Read, 1966), Massachusetts (Manning & Vardaro, 1974), Florida (Volin & McMillan, 1978), Italy (Fiume & Fiume, 2003) and Korea (Kim *et al*., 2003). Corky root is considered a serious problem for early planting of fresh market and processing tomatoes in many production areas of California (McGrath & Campbell, 1983). An important feature of the disease is that the symptoms are hardly noticeable until the root is exposed, except for a decrease in fruit yield and shoot growth (Ebben, 1974).

The demand for organically produced products is increasing all over the world due to growing concerns about food safety and environmental pollution. Organic farming is 'a system that provides healthy food and other products through natural ecological cycles, methods that care for the environment and fair relations with all involved' (IFOAM, 2007). In Sweden, the organic tomato growing area comprises 1.8 hectares, which corresponds to approximately 4% of the total tomato growing area of 45.6 hectares (Statistiska Meddelanden, 2007). Tomatoes have previously been the largest crop in organic greenhouse cultivation, but the area has decreased in the past two years (www.krav.se). Organic tomato growing is spread over southern and central Sweden and is often carried out in small enterprises as a complement to field growing. The tomatoes are mainly sold locally in shops or direct to the consumer, but supply to wholesalers also occurs (C. Winter, pers. comm.).

In organic production systems, farmers rely on preventive, cultural, biological control and integrated methods for disease management. In this regard, plant disease control can be achieved by crop rotation, intercropping, organic manuring and use of resistant cultivars and bio-control agents such as beneficial fungi, bacteria and nematodes. The availability of acceptable resistant cultivars against corky root disease is limited and the current methods for corky root disease management in organic tomato production are inadequate. Therefore, research on corky root disease management by non-chemical methods needs to develop new control methods, in order to increase tomato yields in organic production systems.

Recently, participatory research involving growers has been shown to be a successful step in plant disease management as it encourages local experimentation to determine optimal management strategies (Nelson *et al*., 2001; Pande *et al*., 2001; Ortiz *et al*., 2004). Involving local people as participants in planning and carrying out research can enhance effectiveness and save time and money in the long run (Cornwall & Jewkes, 1995). In this thesis, research work

was carried out using a group of organic tomato growers in Sweden as a participatory research group to develop management strategies regarding corky root disease. The intention was that this participatory approach would serve as a mechanism to ensure that the research work was relevant to the needs and conditions of commercial organic tomato growers.

Aims of the thesis

The overall aim of the thesis was to develop reliable management strategies for corky root disease that could be used by commercial growers in organic tomato production. The underlying hypothesis was that addition of compost and fungivorous nematodes to greenhouse soil would reduce corky root disease infection and, moreover, that sharing knowledge with organic tomato growers would help to develop management strategies for corky root disease. The following questions were addressed:

- Is it possible to suppress corky root disease by the application of compost?
- Can fungivorous nematodes feed, survive and reproduce on *Pyrenochaeta lycopersici*?
- Is it possible to suppress corky root disease by the application of fungivorous nematodes to the soil?
- How do fungivorous nematodes and composts interact in corky root disease suppression?
- How can corky root disease management strategies be developed in participation with organic tomato growers?

Background

Corky root disease

Corky root disease, also known as brown root rot disease (Last *et al.*, 1969), was almost forgotten in the 1960s as tomato production in the greenhouse was then based on inorganic substrates such as rockwool, sand and gravel. In the late 1980s and 1990s, problems with corky root disease reappeared as organic tomato production based on soil substrates increased. The disease has become a serious threat for organic tomato production since the middle of the 1990s. Corky root attacks the root system of the plant (Fig. 1), causing rotting of smaller feeder roots, brown lesions on small roots and typical corky lesions on larger roots (Pohronezny & Volin, 1991). However, it is not known whether corkiness is a response of the plant to the infection or a feature of the pathogen itself (Blancard, 1992). The evolving infection leads to progressive damage of the root system, resulting in disruption of nutrient and water uptake (Goodenough & Maw, 1973). The formation of brown lesions and subsequent loss of fibrous roots at an early stage of growth leads to severe losses in fruit yield (Last & Ebben, 1966). In intensive production systems in Swedish greenhouses where the soil is reused for 3-4 years for tomato cultivation, this disease may cause yield reductions of 30-40%, but

losses of up to 75% have been observed in European greenhouses (Forsberg, Sahlström & Ögren, 1999).

Fig. 1. Infected tomato roots with symptoms of corky root disease.

Causal pathogen Pyrenochaeta lycopersici

Pyrenochaeta lycopersici was first isolated in 1929 but was known as grey sterile fungus until it was identified as *P. lycopersici* in 1966 (Punithalingam & Holliday, 1973). The fungus is found in root lesions and in apparently healthy tissue of plants other than tomato, such as pepper (*Capsicum annum* L.), tobacco (*Nicotiana tabacum* L.), eggplant (*Solanum melongena* L.), melon (*Cucumis melo* L.), cucumber (*Cucumis sativus* L.), spinach (*Spinacea oleracea* L.), squash (*Cucurbita pepo* L.), mad apple (*Datura stramonium* L.) and safflower (*Carthamus tinctorius* L.) (Grove & Campbell, 1987; Shishkoff & Campbell, 1990).

The fungus belongs to the Fungi Imperfecti group, which produce only asexual spores; conidia within pycnidia. Formation of pycnidia by *P. lycopersici* has not been observed under natural conditions (Punithalingam & Holliday, 1973). However, pycnidia of *P. lycopersici* have been produced *in vitro*, on agar medium

at constant exposure to fluorescent cool-white lamps (McGrath & Campbell, 1983). In soil the fungus multiplies by microsclerotia, which are firm structures of hyphal mass (Fig. 2). Factors influencing the germination of microsclerotia of *P. lycopersici* have not yet been fully identified. Germination is probably stimulated by root exudates of the host plant, as is the case in microsclerotia germination of other soil-borne fungi such as *Verticillium dahliae* (Mol & van Riessen, 1995). The mycelia from germinating microsclerotia attack the root system of the plant and cause disease (Fig. 2). In the absence of the host plant, *P. lycopersici* survives in the soil as microsclerotia. The microsclerotia are 63.5 µm x 44.8 µm in size (Grove & Campbell, 1987). An outer skin layer on microsclerotia of *P. lycopersici* has been observed under electron microscope, whereas no such structure has been recorded on the microsclerotia of any other fungus (White & Scott, 1973; Ball, 1979). The longevity of microsclerotia in soil is most likely the result of their external skin, heavy pigmentation (probably melanin) and small size (Ball, 1979). Resistance of the microsclerotia to drying and heat makes it difficult to eradicate this pathogen in the soil (Ebben, 1974). Microsclerotia of *P. lycopersici* can survive in soil for up to 5 years (Termohlen, 1962) but survival can even extend up to 10-15 years (O. Andersson, pers. comm.).

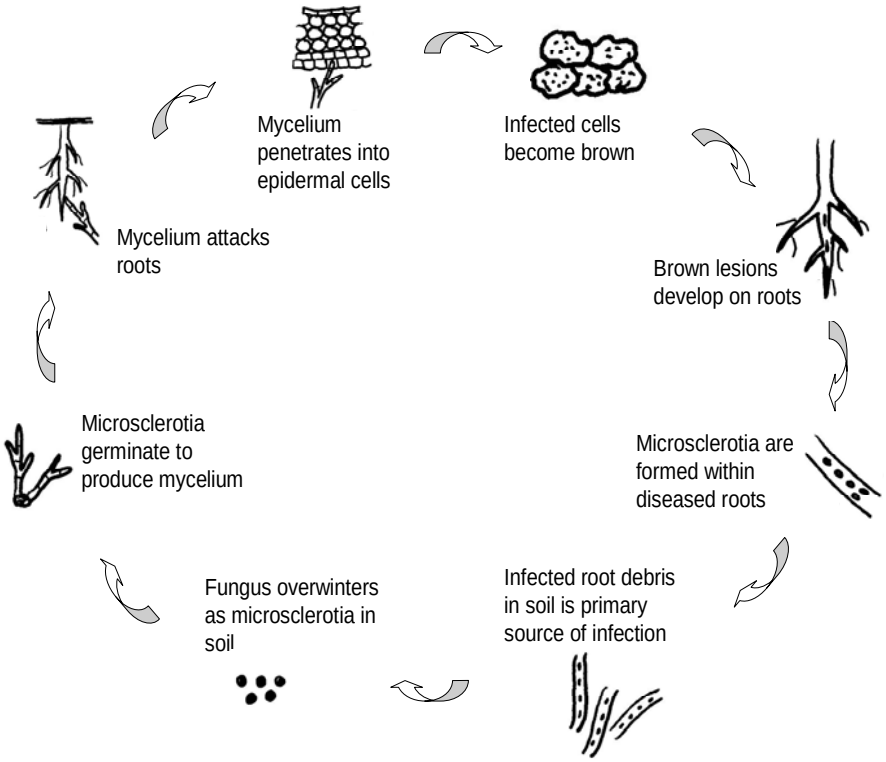

Fig. 2. Life cycle of the corky root pathogen, *Pyrenochaeta lycopersici*.

The fungus is ecologically an obligate parasite with poor competitive ability (Davet, 1976; Shishkoff & Campbell, 1990). The low competitive ability of the pathogen has been suggested to be a likely mechanism of corky root disease suppression by organic amendments, as these stimulate other soil microbiota (Workneh & van Bruggen, 1994a).

Pyrenochaeta lycopersici is a slow-growing fungus, which makes the isolation procedure tedious (Infantino & Pucci, 2005). Once isolated the fungus rarely sporulates in pure culture, and therefore identification of the fungus is difficult in laboratory conditions. These difficulties mean that molecular methods such as PCR-based techniques should be used for rapid and reliable detection of the disease. A PCR-based assay has been suggested as a valid tool for studies on the epidemiology of corky root disease and for the implementation of control strategies (Infantino *et al*., 2003; Infantino & Pucci, 2005).

Management of corky root disease

Chemical control

In conventional tomato production, soil fumigants such as methyl bromide, chloropicrin and methane sodium have been used successfully against corky root disease (Punithalingam & Holliday, 1973; Campbell, Schweers & Hall, 1982; Malathrakis & Kambourakis-Tzagaroulakis, 1989). The chemical treatments are expensive, destroy beneficial soil microorganisms and cause environmental pollution; in particular, methyl bromide has been recognized as an ozone-depleting chemical and is going to be phased out world-wide by 2015 according to the Montreal Protocol (Albritton & Watson, 1992; Ristaino & Thomas, 1997). However, chemical treatments are not allowed in organic production systems.

Cultural methods

Soil solarisation is a method of disinfestation accomplished by covering the soil with transparent polyethylene sheets in order to increase heat before planting, which is effective in corky root management (Moura & Palminha, 1994; Ioannou, 2000). This treatment increases the production costs and has been widely exploited in warm countries where solar radiation is sufficient to create lethal soil temperatures. Steaming of infested soil can reduce corky root incidence but due to the limit of steam penetration there is a risk that the inoculum of *P. lycopersici* will be left in deeper soil layers. For example, the percentage of corky root disease infection on roots has been shown to increase at soil depths of 20 cm after steam treatment (Last *et al*., 1968). Steam sterilisation kills most of living organisms in the soil, including beneficial ones, which is not in agreement with organic production goals (Sorensen & Thorup-Kristensen, 2006). Crop rotation in order to control *P. lycopersici* is not a definitive solution as the fungus has a relatively wide host range (Grove & Campbell, 1987). Use of grafted tomato plants (grafting a commercial cultivar onto a rootstock tolerant to *P. lycopersici*) is another option but it greatly increases planting costs. The taste of tomatoes may be impaired

depending on the rootstock and the grafted variety (K. Sjöstedt, pers. comm.). Tolerant rootstocks can also be attacked when the inoculum level of *P. lycopersici* in the soil is high, but the onset of the disease is delayed (Forsberg, Sahlström & Ögren, 1999). There is a way to reduce the inoculum of *P. lycopersici* by removing the topsoil and replacing it with non-infested soil. However, this is very laborious and there is also a risk that inoculum will be left in the deeper soil, since microsclerotia of *P. lycopersici* develop on infected tomato roots and these roots can penetrate into deeper soil. An alternative to soil replacement is to grow tomato plants in limited growing beds using non-infested soil. This technique provides good control of the effective root volume and also of nutrient leaching from the growing system (Gäredal, 1998).

Plant resistance is an effective and long-lasting control strategy against plant diseases. Unfortunately, commercial cultivars of both processing and fresh market tomatoes are susceptible to corky root disease (Pohronezny & Volin, 1991). The known source of resistance to corky root disease, the 'pyl' gene, has incomplete penetrance and expressivity (Fiume & Fiume, 2003) suggesting that more research is still needed for new genetic resources. Thus the limitations of available methods for controlling corky root disease have stimulated the search for alternative methods.

During the cultivation of tomato, some technical measures are helpful to limit the severity of corky root disease. For example, increasing the size of the propagation pot can increase the amount of healthy roots of tomato seedlings. When seedlings with increased volume of healthy roots are transplanted into infested soil, the onset of the disease in tomato plants can be delayed (Ebben, 1974). Cool temperatures stimulate lesion expansion and symptom development of corky root disease. During the first few weeks of seedling growth, a cool temperature (~16 ºC) probably has a significant effect on increasing disease severity and this effect is not overcome as seasonal warming proceeds (Shishkoff & Campbell, 1990). Early planted tomato seedlings in California were shown to have more disease than late planted seedlings and this was attributed to cool temperatures during the early stages of plant growth (Campbell, Schweers & Hall, 1982). Therefore, avoiding cool temperatures during the early stages of plant growth is important. A well-balanced fertilisation regime during cultivation is also necessary, since a high N content in soil and tomato plant tissues favours the development of corky root disease (Workneh & van Bruggen, 1994a).

Biological control

Biological control is defined as the use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be (Eilenberg, Hajek & Lomer, 2001). Biological control is an alternative approach for dealing with pest problems in both organic and conventional crop production, since the use of pesticides causes various problems that include pollution of the environment, development of resistance in pest populations and effects on non-target organisms.

The antagonistic fungus *Trichoderma* has been shown to inhibit the growth of *Pyrenochaeta lycopersici* in *in vitro* assessments (Whipps, 1987; Vanachter, van Wambeke & van Assche, 1988; Pérez *et al.*, 2002). Pérez *et al.* (2002) found four isolates of *T. harzianum* that were able to inhibit the *in vitro* development of *P. lycopersici* and they suggested the involvement of non-volatile metabolites and extracellular fungal cell wall hydrolysing enzymes in bio-control by *T. harzianum*. Nevertheless, it appears that each *Trichoderma* isolate may react differently to a particular plant pathogen in bio-control activity (Pérez *et al.*, 2002). This difference is due to the ability of an isolate to produce antibiotics and/or to express genes that regulate extracellular fungal cell wall hydrolysing enzymes (Dennis & Webster, 1971a,b; Grondona *et al*., 1997). The antagonistic chemicals produced by *Trichoderma* spp. are degraded very rapidly and therefore a constant presence and active development of the antagonist in the soil is necessary to maintain the expected antagonistic activity (Vanachter, van Wambeke & van Assche, 1988).

In greenhouse trials, bacterial antagonists such as *Bacillus subtilis* and *Streptomyces graminofaciens* have been found to effectively suppress corky root disease of tomatoes and enhance plant growth, resulting in higher yields (Bochow, 1989). The secretion of volatile and diffusible metabolites, but not fungal cell wall hydrolysing enzymes, from *Bacillus subtilis* caused the inhibition of the tomato root fungus *Rhizoctonia solani* in an *in vitro* study reported by Montealegre *et al*. (2003).

The commercial biofungicide Binab TF WP®, based on the antagonists *Trichoderma polysporum* Bisset and *T. harzianum* Bisset, is primarily used in the greenhouse to control soil-borne fungal diseases in tomato, cucumber and flowers and is available in Swedish market (www.binab.se). Another product, Mycostop® (Verdera Oy, Esbo, Finland), a commercial formulation of the antagonist *Streptomyces griseoviridis* strain K61, has proven effective against *P. lycopersici* when applied with irrigation water (Minuto *et al*., 2006).

Plant disease control by composts

Soil amendment with composts is an interesting cultural practice to improve soil fertility as well as to suppress plant diseases. Several studies have reported that composts can suppress soil-borne plant pathogens within genera such as *Fusarium*, *Phytophthora*, *Pythium* and *Rhizoctonia*, where physical, chemical and biological properties of composts play major roles in disease suppression (Chen, Hoitink & Schmitthenner, 1987; Reuveni *et al*., 2002; Diab, Hu & Benson, 2003; Noble & Coventry, 2005; Scheuerell, Sullivan & Mahaffee, 2005; Termorshuizen *et al*., 2006; van Rijn, 2007). However, composts vary considerably in physical, chemical and biological composition and consequently in their ability to suppress soil-borne diseases. Thus, one compost may be highly suppressive to one disease while having little or no effect on other important plant diseases.

The compost-induced disease suppression process is mediated by a three-way interaction involving compost types, plant species and pathogens (van Rijn, 2007). It is generally thought that the rhizosphere microbial community plays a crucial

role in disease suppression by compost. On the other hand, the genetic and functional diversity of the rhizosphere microbial community is dependent on plant species, through the quality and quantity of root exudation (Lemanceau *et al*., 1995; Wieland, Neumann & Backhaus, 2001; Marschner, Crowley & Yang, 2004). Thus compost-induced disease suppression can be related to plant species. A significant interaction between plant species and compost was found in a study of disease suppression by 9 composts of damping-off caused by *Pythium ultimum* for 5 host species (van Rijn, 2007). In an earlier study, Termorshuizen *et al*. (2006) found a significant compost and pathogen interaction for disease suppression of eighteen composts against 7 pathogens. Thus, disease suppression by compost is both pathogen-dependent and host plant-dependent.

The degree of decomposition of organic matter influences the composition of bacterial diversity, as well as the population and activities of bio-control agents in the compost. Thus level of organic matter decomposition in the compost is related to disease suppression (Hoitink & Boehm, 1999). Particle size, nitrogen content, cellulose and lignin content, electrical conductivity (soluble salt content), pH and inhibitors released by composts are known physical and chemical factors of composts that affect disease suppression (Hoitink & Fahy, 1986). *Trichoderma* spp. and *Gliocladium virens* are the most abundant fungal taxa in composts associated with suppression of soil-borne plant pathogens (Nelson, Kuter & Hoitink, 1983; Hoitink & Boehm, 1999; Suárez-Estrella *et al*., 2007). Bacteria present in suppressive composts as effective antagonists include *Bacillus cereus, B. mycoides, B. subtilis, Enterobacter cloacae, E. agglomerans, Flavobacterium balustinum, Pseudomonas aeruginosa, P. fluorescens, P. putida, P. stutzeri* and *Xanthomonas maltophila* (Hoitink & Fahy, 1986; Hoitink & Boehm, 1999). However, previous studies did not determine which of these bacterial populations predominated in suppressive composts and what their relative contributions were.

Addition of compost to the soil strongly influences the soil microflora and may increase microbial biomass (Albiach *et al*., 2000; Perucci *et al*., 2000; Debosz *et al*., 2002; Darby, Stone & Dick, 2006; Pérez-Piqueres *et al*., 2006). The increased biomass in the soil and the microorganisms in the compost contribute to disease suppressiveness through four mechanisms of biological control: (i) Successful parasitism on pathogens by beneficial micro-organisms; (ii) successful competition for nutrients by beneficial micro-organisms; (iii) antibiotic production by beneficial microorganisms; and (iv) activation of disease-resistance genes in plants by microorganisms (induced systemic resistance) (Hoitink & Boehm, 1999). In soil, dormant root pathogen propagules such as sclerotia, chlamydiospores or oospores are stimulated to germinate after addition of organic amendments and lysis occurs in the absence of the host plant (Papavizas & Lumsden, 1980; Whipps, 1997). Similarly, increased microbiota can inhibit germination of propagules by using the nutrients required for germination. Addition of compost has been shown to reduce soil-borne disease infection in tomato plants caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*, *Pyrenochaeta lycopersici*, *Pythium ultimum* and *Rhizoctonia solani* by increasing the activity of bio-control agents in the rhizosphere (Workneh & van Bruggen, 1994a; De Brito Alvarez, Gagné & Antoun, 1995).

The addition of organic matter such as farmyard manure, green manure or compost can enhance populations of earthworms, which can directly consume hyphae and propagules of plant pathogenic soil-borne fungi (Stephens *et al*., 1994). Organic matter addition also favours other soil fauna such as collembolans and mites, which undoubtedly play a role in suppression of soil-borne plant pathogens feeding on fungal hyphae and propagules (Hoitink & Fahy, 1986; Axelsen & Kristensen, 2000; Friberg, Lagerlöf & Rämert, 2005). Fungivorous nematodes were shown to be more abundant in yard waste and wood chip composts suppressive to *Cylindrocladium spathiphylli*, *Fusarium oxysporum* and *Rhizoctonia solani* than in non-suppressive composts (Termorshuizen *et al*., 2006). Therefore, composts rich in fungivorous nematodes may have the ability to suppress plant disease through fungivorous nematodes present in the composts grazing on soil-borne fungi.

There are a few reports of compost amendment increasing the incidence of disease. For example, use of sewage sludge compost increased the incidence of pea foot rot caused by *Fusarium solani* f. sp. *pisi* (Lumsden, Lewis & Milner, 1993). In another study, damping-off disease in eggplant (caused by *Verticillium dahliae*) and cauliflower (caused by *Rhizoctonia solani*) was significantly increased by the application of horse manure compost and yard waste compost, respectively (Termorshuizen *et al*., 2006).

Plant disease control by fungivorous nematodes

Fungivorous nematodes are equipped with a mouth stylet, which they use to penetrate fungal cells and withdraw the cell contents. This kills the fungal cells. The most common genera of fungivorous nematodes in the soil include *Aphelenchus* (Fig. 3), *Aphelenchoides*, *Tylenchus* and *Ditylenchus* (Freckman & Caswell, 1985; Hofman & s'Jacob, 1989). Fungivorous nematodes typically exist at lower density in the soil than bacteriovorous nematodes (Freckman & Caswell, 1985). However, populations of fungivorous nematodes may rapidly increase on a substrate if fungi suitable as food are available (Hofman & s'Jacob, 1989). Fungivorous nematodes feed on different species of soil fungi, including plant pathogenic, saprophytic and mycorrhizal fungi (Freckman & Caswell, 1985; Giannakis & Sanders, 1989; Ruess & Dighton, 1996; Ruess, Zapata & Dighton, 2000; Okada & Kadota, 2003; Okada, Harada & Kadota, 2005). Feeding on different groups of fungi has a different impact on soil ecology. For example, grazing on mycorrhizal fungi destroys the hyphae of these beneficial fungi, resulting in reduced mycorrhizal development, a disadvantage to plants. On the other hand, when a plant pathogenic fungus is a preferred host then disease reduction may occur. Selective grazing by fungivorous nematodes can also affect the outcome of competition between soil fungi (Ruess & Dighton, 1996). However, soil animals often prefer feeding on plant pathogens rather than saprophytic or antagonistic fungi (Lartey, Curl & Peterson, 1986; Friberg, Lagerlöf & Rämert, 2005). One possible explanation for the preference for plant pathogenic fungi is that they often lack the toxic substances that saprophytes produce (Shaw, 1988). Thus, there is an opportunity to combine a fungivorous

nematode and an antagonistic fungus such as *Trichoderma* sp. in biological control. It has proven possible to enhance the control efficacy of damping-off caused by *Pythium* spp. by combined application of *Aphelenchus avenae* and *Trichoderma harzianum* in pot experiments (Jun & Kim, 2004).

Fig. 3. The fungivorous nematode *Aphelenchus avenae* (Length ~ 0.7 mm).

The ability of fungivorous nematodes to control economically important genera of plant pathogenic fungi within genera such as *Fusarium*, *Pythium* and *Rhizoctonia* has been demonstrated in a number of studies (Rhoades & Linford, 1959; Barnes, Russell & Foster, 1981; Rössner & Urland, 1983; Choo & Estey, 1985; Gupta, 1986; Ishibashi & Choi, 1991; Lootsma & Scholte, 1997; Jun & Kim, 2004; Okada, 2006). Addition of *A. avenae* decreased damping-off disease caused by *Rhizoctonia solani* in cauliflower and *Verticillium dahliae* in eggplant (Rämert *et al*., unpublished). To the best of my knowledge, the ability of fungivorous nematodes in suppression of corky root disease of tomato has not been demonstrated previously.

Mass production of *A. avenae* is possible on solid substrates composed of various industrial vegetable/animal wastes (Ishibashi, Ali & Saramoto, 2000). Another advantage is that *A. avenae* can survive desiccation (Crows & Madin, 1975) and therefore it could be preserved, stored and marketed commercially in a dried state.

Aphelenchus avenae and fungivorous *Aphelenchoides* spp. such as *A. composticola* are not known to feed on higher plants (Hooper, 1974; Hesling,

1977). *Aphelenchus avenae* has been found in root tissue of maize, but it was suggested that the nematode was feeding on the invading fungal pathogen *Pyhium arrhenomanes* (Rhoades & Linford, 1959).

In bio-control of *P. lycopersici* with fungivorous nematodes, it should be borne in mind that the pathogen occurs in the soil as microsclerotia that are highly resistant and fungivorous nematodes are unlikely to be able to feed on these until they germinate to produce mycelium (Fig. 2). When the fungal hyphae penetrate into the cortical tissue of host plant roots, nematodes also cannot attack them. Therefore, the time between microsclerotia germination and mycelium penetration into the host plant is crucial for fungivorous nematodes to attack the pathogen.

Multiplication of the nematodes is greatly affected by the species/strains of host fungi. In an *in vitro* experiment, reproduction of *A. avenae* varied with different strains of *Rhizoctonia solani* where some strains supported a tenfold increase in reproduction (Caubel *et al*., 1981). Consequently, the variability due to the influence of nematode species and strains collected from different localities should also be taken into account (Okada, 1995). The type of culture medium may influence the growth of fungal species, which in turn may affect population development of fungivorous nematodes in *in vitro* tests (Okada, Harada & Kadota, 2005).

Participatory research

Participatory research provides a means to obtain qualitative data in the form of local knowledge and local requirements. Such data can then be assimilated and considered in scientific research, and a better approach to technology transfer can be devised (Probst & Hogmann, 2005). Conventional research tends to generate 'knowledge for understanding', whilst participatory research focuses on 'knowledge for action'. In participatory research, the emphasis is on locally-defined priorities and local perspectives (Cornwall & Jewkes, 1995). Involving farmers in the research process increases the chance of success in the generation of appropriate agricultural technology (Rhoades & Booth, 1982). Participatory research enables researchers to collect datasets from a broader range of environments, while for farmers and growers collaboration with the formal research sector offers opportunities for continuing education on crop management (Nelson *et al*., 2001).

Participatory research has been initiated in order to develop organic tomato production in Sweden, where researchers and an advisor/facilitator are working with a group of commercial organic tomato growers (Eksvärd *et al*. 2001; Ögren *et al*. 2002; Eksvärd & Björklund, unpublished). To improve the economic situations of growers, the participatory group worked on practical problems in greenhouse organic tomato production. Lack of knowledge on available plant nutrients need in organic tomato production in Sweden emerged as an important problem. Corky root disease was identified as another common problem for organic tomato production. It became evident that growers require reliable detection methods to identify the corky root pathogen at an early stage of

infection. A need for suitable methods for corky root disease management in Swedish conditions was also identified.

This thesis describes part of the participatory research for developing organic tomato production and its main aim of keeping corky root disease below an economically tolerable threshold level. The knowledge and perspectives of commercial organic tomato growers experiencing problems with corky root disease were not only acknowledged, but also used to develop the framework of the research work presented in this thesis.

Materials and methods

The research presented in this thesis comprised two *in vitro* experiments and four greenhouse experiments, including a trial in the greenhouse of a commercial organic tomato grower. A participatory research was conducted in participation with a group of organic tomato growers in Sweden.

In vitro experiments

Food attraction of fungivorous nematodes

The aim of this experiment was to compare the attraction intensity of fungivorous nematodes to *Pyrenochaeta lycopersici* compared with other soil-borne fungi and to determine whether populations of fungivorous nematodes developed well on this fungus. *Aphelenchus avenae* Bastian and *Aphelenchoides* spp. (a mixture of two species) were used as fungivorous nematodes in the study. The attraction of the fungivorous nematodes to *P. lycopersici* was tested on agar plates along with plant pathogenic fungi (*Botrytis cinerea* Pers., *Rhizoctonia solani* Kühn and *Verticillium dahliae* Kleb.) and saprophytic/antagonistic fungi (*Mortierella hyalina* W. Gams, *Pochonia bulbillosa* Zare & W. Gams and *Trichoderma harzianum* Rifai) as shown in Fig. 4. Population growth of *A. avenae* and *Aphelenchoides* spp. on *P. lycopersici* was tested on agar plates during a six-week period and compared with that on *P. bulbillosa, R. solani, V. dahliae* and *T. harzianum*. The attraction intensity of nematodes to the different fungi tested was determined as number of nematodes present on the mycelium of each of the fungi 24 h after nematode inoculation. Population development of fungivorous nematodes was determined by counting nematode numbers after destructive sampling of five agar plates (for each fungus) once a week after nematode inoculation for a six-week period (for details see Paper II).

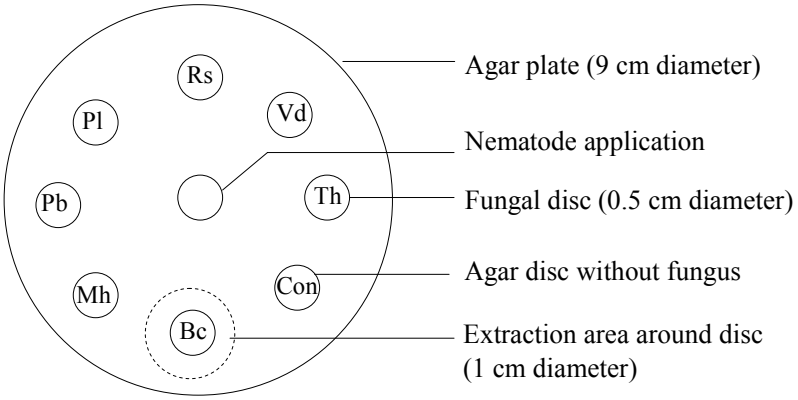

Fig. 4. Schematic presentation of the assay method to determine the attraction of fungivorous nematodes to the test fungi. Nematodes are applied in a hole in the centre and fungal discs are in a ring 2 cm away from the central hole of an agar plate. Bc = *Botrytis cinerea*, Mh = *Mortierella hyalina*, Pb = *Pochonia bulbillosa*, Pl = *Pyrenochaeta lycopersici*, Rs = *Rhizoctonia solani*, Th = *Trichoderma harzianum* and Con = Control (without fungus).

Detection of Pyrenochaeta lycopersici *using PCR method*

Tomato plant materials were collected from four farms (Farms 1-4) of participating growers in central Sweden. On these farms, experiments were performed by participating growers during three years with seven different treatments, with the aim of developing a corky root disease management strategy. The treatments included: A) Mulch with clover-rich green mass; B) mulch with clover-poor green mass; C) mulch with composted animal manure; D) break crop of winter rye (*Secale cereale* L.); E) break crop of hairy vetch (*Vicia villosa* Roth); F) control ungrafted plants; and G) control grafted plants, grafted onto Beaufort rootstock, which is considered resistant to corky root disease (Theodoropoulou *et al*., 2007). The presence of *P. lycopersici* on plant materials from these treatments was detected by a polymerase chain reaction (PCR) -based method developed by Persson, Färeby & Widmark (unpublished) (for details see Paper IV).

Greenhouse experiments

Effects of composts and fungivorous nematodes on corky root disease

The aim of the greenhouse experiments was to evaluate the suppressive effect of composts and fungivorous nematodes on corky root disease and to determine whether the suppressive effect of composts increased with fungivorous nematode enrichment. A further aim was to determine whether composting of *P. lycopersici*-infested soil could reduce disease severity in the infested soil. All experiments were conducted with soil naturally infested with *P. lycopersici* collected from the greenhouse of an organic tomato grower in the vicinity of Uppsala, Sweden. The composts evaluated were a green manure compost prepared from red clover

(*Trifolium pratense* L.), a horse manure compost and two garden waste composts. The composts were mixed with the infested soil (20% vol/vol). Three-week old tomato seedlings (cv. Elin, Weibulls®, Sweden) were transplanted into plastic pots containing 5 l of substrate, with a single seedling in each pot. Fungivorous nematodes (*Aphelenchus avenae* and *Aphelenchoides* spp.) were mass cultured on the fungus *Pochonia bulbillosa* and extracted by the Baermann funnel method (Southey, 1986). Nematode suspension was inoculated (3 or 23 nematodes mL^{-1} substrate for *A. avenae* and 33 nematodes mL^{-1} substrate for *Aphelenchoides* spp.) into the soil and soil-compost mixtures by pouring the nematode suspension into six holes around the seedlings. Inoculation was carried out one day after transplanting of tomato seedlings. Harvesting was conducted ten weeks after seedling transplantation. Disease severity in each plant was evaluated by collecting the following three 3-cm sections of root sample: leaving a segment of 5 cm from the root base and then taking a 3-cm sample, leaving 5 cm and then taking another 3-cm sample, leaving 5 cm and then taking another 3-cm sample. The three root samples from the three distances of each plant were then pooled and mixed. From these root samples, 100 pieces from each plant were examined under a stereomicroscope and grouped into three categories as white (healthy root), light brown (initially infected root) and dark brown (severely infected root). Total fruit weight, shoot and root weight (fresh and dry) from each plant were determined. The final number of nematodes was counted by extracting soil using the method mentioned above (for details see Papers I & III).

To evaluate the effect of fungivorous nematodes in large production systems, *A. avenae* (23 or 50 nematodes mL^{-1} substrate) was inoculated into soil naturally infested with *P. lycopersici* at the greenhouse of a participating grower in Södertälje in southern Sweden (59º12´N, 17º39´E) (for details see Paper IV).

Effect of composting of Pyrenochaeta-*infested soil on corky root disease severity*

In composting of *P. lycopersici*-infested soil, the infested soil, chopped red clover and wheat straw (*Triticum aestivum* L.) were mixed at a ratio of 5:4:1 (dry weight basis). The heap was put outdoors in summer 2004 and was turned over once a week for three weeks to promote aeration and homogeneous conditions. In the beginning of winter 2004, the composted soil was brought indoors and stored at 4 ºC until used in the following summer. A bioassay with the composted soil was conducted in the greenhouse, where corky root disease severity in the composted infested soil was compared with that in non-composted infested soil (for details see Paper IV). Temperature was measured daily in the centre and on the surface of the compost heap until it reached the ambient temperature.

Participatory work with organic tomato growers

With the participation of tomato growers, different management strategies such as use of mulch, break crop, grafted tomato plants, composted *Pyrenochaeta*-infested soil and commercial available bio-control agents based on *Trichoderma harzianum*, *Streptomyces griseoviridis, Gliocladium catenulatum* and *Gliocladium*

spp. against corky root disease were investigated in on-farm and on-station experiments (for details see Paper IV).

The studies presented in this thesis and the experimental systems used are summarised in Table 1.

Table 1. *Summary of studies presented in this thesis and experimental systems used*

Studies	**Systems**	**Papers**			
		I	**II**	**III**	**IV**
Compost effect on corky root disease	Pot soil in the greenhouse	√			
Food attraction of fungivorous nematodes	*In vitro*		√		
Population growth of fungivorous nematodes	*In vitro*		√		
Fungivorous nematode effect on corky root disease	Pot soil in the greenhouse, Limited bed soil in a grower's greenhouse			√	√
Participatory work with organic tomato growers: mulch, break crops, composted infested soil, grafted tomato plants, bio-control agents and PCR method.	On-farm experiments (soil in growers' greenhouses) and on-station experiments (*in vitro* and pot soil in the greenhouse)				√

Statistical analysis

To model the probabilities of healthy, initially infected and severely infected roots, a generalised linear model for ordinal scaled observations was fitted with the procedure GENMOD in SAS (SAS Institute Inc., Cary, NC, USA). The logit link was used and overdispersion within the root was modelled with the option DSCALE. For disease severity in different treatments, the analysis was made with the treatments as explanatory factors (Paper I) and the model was a factorial design with nematode and compost as main effects (Paper III). CONTRASTS were used to separate different treatments. For the relationship between corky root disease severity and biotic and abiotic properties of soil and soil-compost mixtures, the analysis was carried out with the properties as continuous explanatory variables. Microbial population densities (colony numbers of copiotrophic and oligotrophic bacteria, actinomycetes and fungi), nematode numbers and basal respiration were analysed by ANOVA in Minitab (version 14) and treatment differences were compared by least significant difference (LSD) testing at $p<0.05$.

Nematode numbers in the attraction test were analysed using 'proc mixed' in SAS with initial nematode numbers and fungal species as fixed classification variables, and extracted nematode numbers and plates as random classification variables. Initial nematode numbers were included as continuous covariates in the model. *Post hoc* comparisons were tested with least squares means. Nematode numbers in population growth tests were analysed using ANOVA in SAS to determine significant differences between fungi within each week and significant differences between weeks within each fungus. The Bonferroni t test at α = 0.00037 was used to calculate Least Significant Difference (LSD) for pairwise comparisons.

Results and discussions

Effect of composts

Among the composts tested, one garden waste compost (GC1) reduced corky root disease severity, whereas horse manure compost increased the disease. The two other composts, the green manure compost and garden waste compost 2 (GC2), had no effect on the disease (Paper I). The finding that the corky root pathogen *Pyrenochaeta lycopersici* responded differently to different composts is supported by earlier studies where a plant pathogenic fungus behaved differently to different composts during the evaluation of eighteen composts against 7 different pathosystems (Termorshuizen *et al*., 2006) and the evaluation of 12 composts against 5 pathosystems (van Rijn, 2007). Different mechanisms underlying compost-induced disease suppression for different pathosystems were also suggested by Scheuerell, Sullivan & Mahaffee (2005). As compost characteristics, both physiochemical and biological, vary among different composts, disease suppression of different composts against a pathogen may vary as well.

Ammonium nitrogen (NH_4-N) is known to increase several root rot diseases caused by *Fusarium, Phytophthora* and *Rhizoctonia* (Das & Western, 1959; Weinhold, Bowman & Dodman, 1969; Weinhold, Dodman & Bowman, 1972; Huber & Watson, 1974; Nasir, Pittaway & Pegg, 2003). Disease severity caused by soil-borne plant pathogens greatly depends on plant exudates such as amino acids, simple sugars, glycosides, organic acids, vitamins, enzymes, alkaloids, nucleotides and inorganic ions (Reddy, 1980; El-Hamalawi & Erwin, 1986; Davis *et al.*, 2007). Ammonium nitrogen increases the amount of amino acids such as glutamine and asparagine in host plants and is thus thought to increase the level of disease severity, as host exudates are likely to be an important source of nutrients for microorganisms, including plant pathogens, and provide an atmosphere conducive to successful parasitism (Weinhold, Bowman & Dodman, 1969; Weinhold, Dodman & Bowman, 1972; Reddy, 1980; Brown & Hornby, 1987). In contrast to this, however, there are a number of other reports stating that ammonia is toxic to several soil-borne plant pathogens and thus reduces disease severity (Tsao & Oster, 1981; DePasquale & Montville, 1990; Tenuta & Lazarovits, 2002; Zhou & Everts, 2004). In the present study, corky root disease severity increased

with increasing NH_4-N concentration in the growing substrate (Paper I). Therefore, lower amounts of NH_4-N in soil amended with garden waste compost 1 might be a reason for the lower disease severity in this soil. Similarly, higher amounts of NH_4-N in the soil amended with horse manure than in the other three compost-amended soils might have caused the higher disease severity in this soil.

In the present thesis study, it was found that corky root disease severity decreased with increasing concentration of calcium (Ca) in the growing substrate (Paper I). Calcium increases plant cell rigidity and thus helps plants to resist certain enzymes of pathogenic fungi that are used to degrade plant cell walls (Conway & Sams, 1984; Tobias *et al*., 1993; Nigro *et al*., 2006). The high concentration of Ca found in soil amended with garden waste compost 1 provides another possible explanation for the lower disease severity in this soil. Total carbon content was low in the suppressive garden waste compost-amended soil and therefore competition between microorganisms for the limited energy source could have been high in this soil. *Pyrenochaeta lycopersici* might be suppressed in such a competitive situation, as it is known as a weakly competitive fungus (Davet, 1976).

Addition of inorganic nutrients equivalent to 20% green manure compost and the suppressive garden waste compost in the infested soil caused higher disease than the respective compost-amended soils. This indicates the involvement of biotic properties of these two composts in disease suppression. Incorporation of the suppressive garden waste compost into the infested soil increased the number of copiotrophic bacteria and actinomycetes (Paper I). In an earlier study, significant disease suppression of corky root disease had been found to be correlated with increased number of fluorescent *Pseudomonas* (a copiotrophic group) and cellulolytic actinomycetes in the rhizosphere of tomato plants (Workneh & van Bruggen, 1994b). However, increased number of copiotrophic bacteria and actinomycetes was not significantly related to disease reduction in this thesis (Paper I). It might be that the effect of increased number of microorganisms was not detectable during the short duration of the greenhouse experiments.

Workneh *et al.* (1993) found that increased microbial activity in soil caused by the addition of organic amendments reduced corky root disease severity in tomato. In the present study, although microbial activity (measured as basal respiration) of the infested soil was significantly increased by the addition of green manure compost (Paper I), there was no effect of this compost on disease reduction. The analysis of total plant nutrients of the substrates showed that soil amended with green manure compost contained higher levels of NH_4-N than the two soils amended with garden waste compost (Paper I). Thus, disease reduction by high microbial activity in soil amended with green manure compost could have been counteracted by the high ammonium level.

Effect of fungivorous nematodes

Populations of *Aphelenchus avenae* and *Aphelenchoides* spp. developed well on the culture of *Pyrenochaeta lycopersici*, although this fungus was not the most attractive one to the fungivorous nematodes in the attraction test comparing it with other plant parasitic and saprophytic fungi (Paper II). In biological control, the ideal situation would be for pathogenic fungi to be the most attractive to fungivorous nematodes and also the most suitable for their multiplication. The attraction test results showed that the pathogenic fungus *Verticillium dahliae* was more attractive to *A. avenae* than the other fungi tested. In the population growth test, although populations of *A. avenae* and *Aphelenchoides* spp. increased initially on *V. dahliae*, nematode numbers were subsequently higher on *P. lycopersici* and *Pochonia bulbillosa* than on *V. dahliae*. The results from the attraction test and population growth test indicate that for fungivorous nematodes, the suitability of a fungus as a host does not always correspond to the attraction intensity of the fungus. These results are in agreement with previous findings (Townshend, 1964; Ruess, Zapata & Dighton, 2000).

The observation that populations of *A. avenae* and *Aphelenchoides* spp. developed well on *P. lycopersici*, a plant pathogenic fungus, is in line with findings reported by Mankau & Mankau (1963), where plant parasitic fungi such as *Pyrenochaeta* sp., *Rhizoctonia solani* and *Verticillium albo-atrum* proved to be good hosts for *A. avenae* in a population development test on agar plates. However, the present study showed that the fungivorous nematodes also developed well on the saprophytic fungus *P. bulbillosa*. This is contradictory to an earlier report, where meagre populations of *A. avenae* and *Aphelenchoides saprophilus* were found on the saprophytic fungi *Agrocybe gibberosa* Fr., *Chaetomium globosum* Kunze and *Mucor hiemalis* Wehmer (Ruess & Dighton, 1996). In our experiments, *A. avenae* and *Aphelenchoides* spp. were initially cultured on *P. bulbillosa* for mass production, which might have influenced the nematodes to increase their populations on this fungus.

In the population growth test, nematode numbers started to decrease on the antagonistic fungus *Trichoderma harzianum* after week 3 (Paper II). *Trichoderma* spp. are known to suppress plant parasitic nematodes (Windham, Windham & Pederson, 1993; Rao, Reddy & Nagesh 1998; Sharon *et al*., 2001). Egg hatching of the root knot nematode *Meloidogyne incognita* was shown to be reduced by a trypsin-like protease isolated from *T. harzianum* CECT 2413 (Suarez, Rey & Castillo, 2004). This metabolite can also be toxic for fungivorous nematodes. However, it has been reported that large quantities of secondary metabolites of antagonistic fungi are not produced during normal vegetative growth, but occur in circumstances where mycelial growth has ceased (Faull, 1988). It is possible that the colony of *Trichoderma* spp. was favourable for nematodes to multiply at the beginning of the test but afterwards the nematode population started to decline due to the production of toxic compounds as a defence mechanism by this antagonistic fungus.

As populations of *A. avenae* and *Aphelenchoides* spp. developed well on *P. lycopersici* on agar plates, further studies were carried out to evaluate the effect of these fungivorous nematodes on *P. lycopersici* in the soil environment. The fungivorous nematodes were added to pot soil naturally infested with *P. lycopersici*. In greenhouse experiments, *A. avenae* reduced corky root disease severity in the infested soil (inoculation rate 3 or 23 mL^{-1} substrate), while *Aphelenchoides* spp. did not (Paper III). *Aphelenchoides* spp. perhaps changed their food preferences temporarily in the soil environment and selected other soil fungi as their food source. Fungivorous nematodes have the ability to switch between food sources in the soil (Ikonen, 2001). This ability is a strategy to avoid undesirable toxic chemicals in the food source, since changing diet may keep the concentration of toxic chemicals within acceptable limits (Ruess, Zapata & Dighton, 2000).

In greenhouse experiments, *A. avenae* and *Aphelenchoides* spp. failed to maintain their initial population level at the end of experiments (Paper III). The greenhouse experiments continued for ten weeks and therefore the limited amount of substrate available was perhaps not sufficient to supply food for fungivorous nematodes for this longer period. However, the involvement of some other factors such as natural enemies in the soil and/or abiotic factors cannot be excluded.

In the experiment to observe population development pattern of *A. avenae* in the soil, the initial number of nematodes (23 nematodes mL^{-1} substrate) decreased to 5 nematodes mL^{-1} substrate 5 days after inoculation. Nematode numbers decreased further to 3 nematodes mL^{-1} substrate after 10 days of inoculation, but afterwards increased significantly to 6 nematodes mL^{-1} substrate after 15 days of inoculation. There was then a continual decrease in nematode numbers until the end of the experiment, when the population of *A. avenae* was 3 nematodes mL^{-1} substrate. (Paper III). A possible explanation for the quick decline of initial nematode numbers 5 days after inoculation is the change of growing environment for the nematodes which might be a shock for them. However, the increase in nematode population from 3 to 6 nematodes mL^{-1} substrate after 10 days of inoculation could be related to the availability of mycelium after germination of microsclerotia of *P. lycopersici*, although the duration of *P. lycopersici* microsclerotia germination is not yet known. In greenhouse experiments, the final number of fungivorous nematodes in the infested soil was approx. 3 nematodes mL^{-1} substrate, regardless of whether the initial inoculation number was 3 or 23 nematodes mL^{-1} substrate (Paper III). A certain population density at which the food available in the soil is just enough to maintain that population is termed the equilibrium density (Seinhorst, 1966). This indicates that the equilibrium density of the experimental soil was 3 nematodes mL^{-1} substrate. This low number of nematodes was probably sufficient to reduce corky root disease, since a significant disease reduction was observed in the experiments compared with the control without nematode addition (Paper III).

Fungivorous nematodes were added to the infested soil along with compost to enhance the suppressive effect of compost on corky root disease. However, disease reduction did not occur in the treatment where nematodes and compost

were applied together. On the other hand, fungivorous nematodes had a disease reduction effect when applied to the infested soil without compost (Paper III). The final number of fungivorous nematodes in compost-amended soil was not significantly different from that in non compost-amended soil (Paper III). It seems that addition of organic amendments did not help to increase the population of fungivorous nematodes. In general, populations of fungivorous and bacteriovorous nematodes increase after addition of organic amendments to soil (Freckman, 1988; Bulluck, Barker & Ristaino, 2002). Bacteriovorous nematodes increase since the bacterial populations that provide their food base are greater after application of organic amendments (Ferris, Venette & Lau, 1996; Bongers & Ferris, 1999). Addition of organic amendments to soil has also been shown to increase fungal population density in other studies (Mabuhay, Nakagoshi & Isagi, 2006; Pérez-Piqueres *et al*., 2006). However, in this thesis study, addition of compost to the soil did not increase the fungal population density (Paper I). Since compost amendment did not increase fungal density, it is logical that population density of fungivorous nematodes did not increase either.

Addition of *A. avenae* into the infested soil of a grower's greenhouse did not reduce corky root disease severity (Paper IV). In this greenhouse experiment, 85% infection with corky root disease was observed on infected plants, which indicated that the soil was heavily infested with *P. lycopersici*. The fungivorous nematode numbers applied might not have been sufficient to reduce disease in soil with such a high infestation rate of *P. lycopersici*. Klink & Barker (1968) found that the number of *A. avenae* needed for efficient biological control of *Fusarium oxysporum* was directly related to the fungal inoculum level.

Detection of *Pyrenochaeta lycopersici* using PCR method

The infection rate of analysed roots was very low for Farm 1 in 2004, when the soil of the greenhouse was replaced by non-infested soil (Paper IV). However, in the following year, nearly all the plants analysed on this farm showed infection, indicating a rapid recontamination of pathogen-free soil. The results from PCR analyses made the participatory group aware of how fast the corky root pathogen could infest the soil. PCR analyses showed that roots from tomato plants grafted onto Beaufort rootstock contained infection on all farms (Paper IV). Beaufort rootstock, which is considered a resistant rootstock, may therefore multiply the pathogen.

PCR analysis was also used to verify the visual scoring of corky root symptoms on roots of tomato plants. The light (initially infected) and dark brown (severely infected) roots showed clear positive results, while the majority of the white roots (healthy) showed negative PCR reactions. However, 10% of the white roots tested showed positive reactions and therefore these samples were infected without displaying symptoms. Thus PCR analysis can help tomato growers to identify corky root disease at an early stage of infection, which is not possible using the naked eye. However, PCR analysis does not quantify infection but simply gives a positive or negative answer, infected or not infected. Nevertheless it is important

to verify an infection with sometimes confusing symptoms and to be able to detect the pathogen even before the symptoms have developed.

Effect of composting of *Pyrenochaeta*-infested soil on corky root disease

Composting the *Pyrenochaeta*-infested soil with fresh red clover did not reduce corky root disease severity (Paper IV). In general, soil-borne plant pathogens are inactivated by the heat produced during the thermophilic phase of the composting process (Bollen, 1985; Ryckeboer, 2001). However, survival of a few soil-borne plant pathogens such as *Fusarium oxysporum* f. sp. *lycopersici, Macrophomina phaseolina*, *Plasmodiophora brassica* and *Polymyxa betae* during composting has been reported (Noble & Roberts, 2004; van Rijn, 2007). Heat was considered the sole factor causing eradication of *Verticillium dahliae* during composting where the internal temperature of the compost heap was 57-70 ºC (Bollen, 1985). *Pyrenochaeta lycopersici* exists in the soil as microsclerotia, as does *V. dahliae*. In the present study, the internal temperature of the compost heap was around 55 ºC for two days (Paper IV). Despite this, corky root disease severity was higher in composted infested soil compared with non-composted infested soil (Paper IV). This indicates that the current composting process did not eradicate *P. lycopersici*. Moreover, a higher concentration of NH_4-N in the composted infested soil (7 mg kg^{-1} dw) than in the non-composted infested soil (3 mg kg^{-1} dw) probably caused higher disease incidence in the former. Increased concentration of NH_4-N in the substrate was shown to favour corky root disease severity (Paper I).

During discussion in participatory research group, the growers suggested that the composted soil could be kept outdoors for the whole winter period. This suggestion could be considered because chilling damage may account for decreased germinability of microsclerotia of *P. lycopersici*. A previous study has shown an indication of low temperatures inducing inhibition of microsclerotia germinability in soil-borne fungi (Roth, Griffin & Graham, 1979). Lower numbers of germinable microsclerotia of *Cylindrocladium crotalariae* were found in a naturally infested soil incubated at -3 ºC than at 5 ºC, while no germinable microsclerotia were found for soils incubated at -10 ºC. In this thesis study, the infested soil was composted outdoors for 5 months and subsequently stored in a cold room (4 ºC). The aim was to determine the disease reduction effect of composting the infested soil. Therefore, the composted soil was taken indoors before winter in order to escape the chilling effect on disease reduction.

Participatory work with organic tomato growers

The tomato growers viewed mulching as an interesting alternative for corky root disease management, since the mulch layer promoted root development and since other benefits, such as decreased evaporation from plant beds and inhibition of weed growth, are achieved through mulching. The growers who participated intended to continue mulch treatment, as it has become part of their accepted method. The growers found that it was difficult to evaluate some methods such as

use of break crop within a limited period and they believe that break crops may give greater effects in the longer term.

The effects of bio-control agents on corky root disease control were studied in a student's project by Rita Varela at Swedish University of Agricultural Sciences. In that study, the antagonists tested showed good inhibition of *P. lycopersici* in *in vitro* tests and in the greenhouse experiment all treatments except the standard treatment with *Gliocladium catenulatum* (Prestop WP®) had more white roots (healthy roots) compared to the control (R. Varela, pers. comm.). However, the fact was that these antagonists showed better inhibition of *P. lycopersici* in nutrient-rich medium than in nutrient-poor medium in *in vitro* tests. Therefore, it seems that in the soil environment, the antagonists will need extra nutrients to improve their ability as bio-control agents against *P. lycopersici*. Under nutrient-poor conditions, germination rate, hyphal extension and sporulation of *Trichoderma* isolates are reduced and the bio-control ability of this fungus is reduced (Beagle-Ristaino & Papavizas, 1985; Nelson, Harman & Nash, 1988; Hjeljord & Tronsmo, 1998; Hjeljord, Stensvand & Tronsmo, 2001). The same suggestion is given by BINAB Bio-Innovation AB, the manufacturer of Binab TF WP®: 'when the product is applied to plants, *Trichoderma* propagules become active as the formulated product contains a 'food package' but under certain circumstances it is necessary to enhance the growth by adding sugar' (www.binab.se). Therefore, during application of Binab TF WP®, addition of exogenous nutrients to soil might be helpful in improving the degree of disease control. Introducing the antagonists into soil prior to transplanting of tomato seedlings should also be considered. Prior application and nutrient activation will ensure a good colony of the antagonists in limited bed soils before the plant makes contact with *P. lycopersici*.

The participatory group agreed that they cannot rely on just one measure to slow down the growth of *P. lycopersici* and therefore, integration of different measures is required to maximise corky root disease control.

At the end of the study, the participatory research work was evaluated. Growers were asked to respond to seven questions by making a tick on a scale of 1 (very negative) to 5 (very positive). All seven questions were given a positive response of between 3.5 and 5 (Paper IV). The growers viewed this participatory work as an opportunity to exchange information with each other and with the researchers and were interested in continuing the process.

Concluding remarks and future perspectives

- A compost with a low concentration of ammonium nitrogen and a high concentration of calcium reduced corky root disease severity (Paper I).
- For biological control purposes, matching fungivorous nematodes to the fungus host is crucial. *Aphelenchus avenae* and *Aphelenchoides* spp. multiplied well on *P. lycopersici* culture *in vitro*, indicating that the fungus is a good host for these fungivorous nematodes (Paper II).
- Fungivorous nematodes (*A. avenae*) reduced corky root disease severity when added to *P. lycopersici*-infested soil (Paper III).
- Disease reduction did not occur after combined application of composts and fungivorous nematodes to infested soil (Paper III).
- PCR analysis can identify corky root disease at an early stage of infection, which is not possible using the naked eye. Beaufort rootstock, which was considered a resistant rootstock to corky root disease, showed infection by PCR analysis (Paper IV).
- Composting of *P. lycopersici*-infested soil did not reduce corky root disease severity (Paper IV).
- Commercially available bio-control agents showed good *in vitro* inhibition of *P. lycopersici* and reduced corky root disease severity in greenhouse trials. Activating the antagonists with nutrients during application might be helpful to improve the degree of control (Paper IV).
- In this study, no single treatment showed such a high degree of control of corky root disease that it could be recommended to growers. Therefore, integration of different methods is necessary in order to improve the degree of control.

There is still a considerable lack of information on the biology and ecology of the corky root pathogen *Pyrenochaeta lycopersici*, e.g. factors influencing germination of *P. lycopersici* microsclerotia and susceptible stages in the life cycle of the pathogen in which antagonistic fungi or other soil organisms can attack. Knowledge about these aspects is important for better understanding of the interaction between *P. lycopersici* and other organisms in soil and ultimately for optimal biological control. The results from this study do not provide information about the appropriate time for inoculation of fungivorous nematodes into soil. Future studies should determine the inoculation time of fungivorous nematodes in relation to the development of the plant and that of *P. lycopersici*. The finding from this study that fungivorous nematodes reduced corky root disease severity in pot experiments would be strengthened if the presence of *P. lycopersici* could be detected in the intestine of fungivorous nematodes inoculated into soil. I regard detection of *P. lycopersici* in the intestine of fungivorous nematodes by quantitative real time PCR methods as being of special interest for future study.

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