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Effect of soil amendments and biological control agents (BCAs) on soil-borne root diseases caused by *Pyrenochaeta lycopersici* and *Verticillium albo-atrum* in organic greenhouse tomato production systems

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Abstract The effect of different soil amendments and biological control agents on soil-borne root diseases that cause significant economic losses in organic and other soil-based tomato production systems *(Pyrenochaeta lycopersici* and *Verticillium albo-atrum*) was compared. Organic matter inputs (fresh *Brassica* tissue, household waste compost and composted cow manure) significantly reduced soil-borne disease severity (measured as increased root fresh weight) and/or increased tomato fruit yield, with some treatments also increasing fruit number and/or size. Soil biological activity also increased with increasing organic matter input levels and there were significant positive correlations between soil biological activity, root fresh weight and fruit yield. This indicates that one mechanism of soil-borne disease control by organic matter input may be increased competition by the soil biota. Chitin/chitosan products also significantly reduced soil-borne disease incidence and increased tomato fruit yield, number and/or size, but had no effect on soil biological activity. Biological control products based on *Bacillus subtilis* and *Pythium oligandrum* and commercial seaweed extract (Marinure) and fish emulsion (Nugro)-based liquid fertilisers had no positive effect on soil-borne disease incidence and fruit yield, number and size. The use of 'suppressive' organic matter inputs alone or in combination with chitin/chitosan soil amendments can therefore be recommended as methods to control soil-borne diseases in organic and other soil-based production systems.

Keywords Soil amendments · Biological control agents · *Pyrenochaeta lycopersici* · *Verticillium albo-atrum* · Organic tomato production

Abbreviations

CCM	Composted cow manure
CMC	Controlled microbial composting

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FBT	Fresh Brassica tissue
HWC	Household waste compost
RFW	Root fresh weight
BCA	Biological control agent
RCBD	Randomised complete block design
DH	Dehydrogenase activity
INTF	Iodonitrotetrazolium formazan
SBA	Soil biological activity

Introduction

The main soil-borne diseases in organic and other soil-based tomato production systems are corky root rot, caused by *Pyrenochaeta lycopersici* and verticillium wilt, caused by *Verticillium* spp. (especially *V. dahliae* and *V. albo-atrum*). Corky root rot is more severe in regions with cooler climates such as countries in northern Europe, with yield losses of up to 75% reported (Ebben et al. 1978; Campbell et al. 1982). However, it can also cause problems in southern European countries especially in glasshouse crops during the winter months and in early open-field tomato crops (Pegg and Brady 2002). The pathogen, which also attacks a wide range of other glasshouse crops including pepper, aubergine, cucumber, squash, and melon, is very persistent in soil and therefore cannot be controlled by break-crops or rotation of crops in the greenhouse (Ebben et al. 1978; Grove and Campbell 1987). *Verticillium* spp. also cause significant problems in tomato crops throughout Europe; *V. albo-atrum* is more prevalent in cooler climates, and *V. dahliae* is more destructive in warmer climates (Pegg and Brady 2002).

Soil-borne diseases in greenhouse crops are currently mainly controlled by chemical soil disinfection (e.g. methyl bromide) in conventional systems and soil steaming in organic greenhouse production systems (Pegg and Brady 2002). However, there is increasing pressure to replace both chemical and steam-based soil disinfection because both methods have been linked to significant environmental and/or potential human health problems. For example, methyl bromide is known to contribute to the depletion of the atmospheric ozone layer and this in turn is known to increase UV-B irradiation and the risk of skin cancer and cataract incidence in humans (Van Loehnen et al. 2003; Workneh and Van Bruggen 1994). Also, steam soil treatments have been shown to significantly increase energy/fuel use in glasshouse production and both steam and chemical soil disinfection treatments have been shown to negatively affect beneficial soil microflora and fauna (Van Loehnen et al. 2003; Bennett et al. 2003). As a result, there is significant consumer, supermarket and legislative pressure to phase out methyl bromide in conventional production and reduce the use of soil steaming for the control of soil-borne diseases in organic and other soil-based production systems (Van Loehnen et al. 2003).

A range of alternative soil treatments have been suggested as potential replacements for chemical and thermal soil disinfection. These include (a) organic matter-based soil amendments (animal and green manures, and/or composts made from organic waste), (b) biological control agents (BCAs) and (c) plant/seaweed extracts and/or other compounds (e.g. chitin) which have been linked to 'plant strengthening' or 'induced resistance' effects (Noble and Coventry 2005; Xiao et al. 1998; Matthiessen and Kirkegaard 2006).

Application of organic matter inputs (animal and green manures, composts) has been shown to suppress a range of soil-borne diseases (Noble and Coventry 2005). This is thought to be due to a range of mechanisms including **(a)** enhanced competition and antagonism by the soil biota associated with increased microbial activity in soil, **(b)** induced resistance in roots from compounds in the organic matter and/or **(c)** release of antimicrobial compounds from the organic matter (Xiao et al. 1998; Noble and Coventry 2005.). The disease suppressive effect of incorporating Brassica green manures, crop residues and/or processing waste into soil has been linked to their glucosinolate contents (Sarwar and Kirkegaard 1998; Kirkegaard and Sawar 1998; Xiao et al. 1998; Matthiessen and Kirkegaard 2006). Hydrolysis of glucosinolates present in Brassica tissue in soil was shown to result in the release of isothiocyanate, and a biofumigation effect on a range of soil-borne pathogens (Morra and Kirkegaard 2002; Bailey and Lazarovits 2003; Matthiessen and Kirkegaard 2006). The use of Brassica tissue based organic matter inputs may therefore result in additional suppressive effects compared to other organic matter inputs (e.g. green waste or cattle manure compost).

Changes in overall soil biological activity (SBA) associated with organic matter inputs may also increase the rate of mineralisation of soil organic matter. This is thought to be of particular importance in organic tomato farming systems, where readily-available mineral N and P fertilisers are prohibited, and organic matter-based fertility inputs (animal and green manures and composts made from organic wastes) are the main fertility source (Lampkin 2002). Soil treatments such as soil steaming, which reduce SBA, are therefore undesirable because they not only reduce the competition/antagonism potential in soil, but also the rate at which nitrogen and other plant nutrients are mineralised and become available to the plant.

There are a range of reports of biological control for *P. lycopersici* and *Verticillium* spp. by fungal, oomycete and bacterial antagonists (Bochow 1989; Pegg and Brady 2002). Certain *Trichoderma* strains have been shown to have antagonistic activity against *Pyrenochaeta lycopersici* (Vanachter et al. 1998) and a range of fungi (*Trichoderma*, *Gliocladium* and *Penicillium*) have been described as potential BCAs for *Verticillium* spp. (Pegg and Brady 2002). More recently, the oomycete *Pythium oligandrum* has been described as providing control of *Verticillium* and other soil-borne diseases (Benhamou et al. 1999). Pre-planting applications to roots of antagonistic strains of *Bacillus subtilis* and *Streptomyces graminofaciens* have also been reported to reduce corky root rot disease symptom development and enhance crop growth rates and yields in soils infested with corky root rot (Bochow 1989). For *V. albo-atrum* there are numerous studies showing effective control by *B. subtilis* (Podile et al. 1985)

Applications of plant/seaweed extracts and compounds such as chitin have also been linked to reductions in soil-borne disease incidence, but little information is available on their efficacy against soil-borne diseases in tomato and/or their mode(s) of action (Vruggink 1970; Sneh et al. 1971).

Although the potential of several alternative approaches has been demonstrated, there is a lack of studies comparing the **(a)** relative efficacy of different soil amendment types (BCAs, elicitors and organic matter-based soil amendments) and levels, **(b)** relative activity of alternative treatments against different types of soil-borne disease inocula and **(c)** effect of different soil amendments on SBA. These gaps in knowledge have prevented the integration of alternative control methods into both organic and soil-based conventional production systems. The main objectives of the work described here were therefore: to compare the effects of alternatives (BCAs, elicitors, soil organic matter inputs) to soil disinfection for the control of corky root rot, on root disease development and tomato fruit yield and quality characteristics (experiment 1), to study the effect of soil treatments against different soil pathogen inoculum types (corky root rot alone and mixed inocula of corky root rot and *Verticillum albo-atrum*) (experiment 2), to identify dose–response relations for selected soil amendments (experiments 2 and 3), to identify potential additive effects of combined use of different soil amendments (experiment 3), to quantify the effect of soil amendments on SBA (dehydrogenase activity) (experiment 3).

Materials and methods

Tomato variety and soil pathogen inocula

Non-grafted plants of the hybrid variety ‘Star fighter’ (Bruinsma seeds, NL) were used in all experiments. Seedlings were supplied by a commercial organic glasshouse production company (Cantelo Nurseries Ltd., Cantelo, Somerset, UK) and transplanted into pots when approximately four weeks old. The variety produces uniformly round fruit, has no reported tolerance/resistance to *P. lycopersici* and *Verticillium* spp., and is widely used in the UK and the Netherlands as a salad tomato variety. Three experiments were conducted to examine the effects of soil treatments individually (experiments 1 and 2) and in combination (experiment 3) on tomato production in soils infested with *P. lycopersici* only (experiment 1 and 2) or with a mixture of corky root rot and *V. albo-atrum* (experiments 2 and 3).

Source of potting soil

Soil used in all three experiments was collected from a commercial organic glasshouse tomato-production company (Cantelo Nurseries Ltd.). Corky root rot was the only pathogen that could be detected in soils, by visual examination of roots and vascular systems of

tomato plants, and standard mycological testing of 50 root systems from plants grown in the greenhouse prior to the collection of soils. To test for the presence of *Fusarium*, vascular tissues were examined for symptoms of *Fusarium* and *Verticillium* infection by LP and P microscopy. In addition, tissue samples from the stem-base of plants were taken, surface-sterilised (5 min in a 5% solution of Domestos, a commercial hypochlorite product) and then plated onto potato dextrose agar (PDA) (with and without lactic acid) and semi-selective media for *Fusarium* spp. (Komada 1975). The absence of *Verticillum* inocula in soil was confirmed by plating soil adhering to plant roots onto semi-selective media for *Verticillum dahlia* (NP10) and *V. albo-atrum* using established methods (Easton et al. 1969; Sorensen et al. 1991). *Fusarium* and *Verticillium* could not be isolated from any of the plants or nursery soils, respectively. Since all plants showed clear symptoms of corky root rot, the presence of *P. lycopersici* was confirmed by visual assessment of roots only. The soil collected in different parts of the glasshouse was mixed and homogenised using a concrete mixer to minimise differences in substrate structure, texture and inoculum density and then placed into 15 l pots (the soil volume was similar to that used for tomato plants in the commercial organic production unit where soil was collected).

Soil treatments

A summary of the treatments used in each experiment is given in Table 1. The composted cow manure (CCM) came from an organic farm (Tio Ltd., Inverness, UK) and the 50% amendment level represents the standard volume of compost added to soil in commercial organic tomato glasshouse production systems immediately after conversion to organic management. CCM was produced using a windrow-based system and a controlled microbial composting (CMC) protocol without compost inoculants (Lübke 2000; Litterick et al. 2003; Rees 2007). The process takes approximately 2 months and involves regular turning (depending on CO_2 and temperatures measured in the windrow. Compost is turned five to seven times during the heating-up phase in week 1 and then once or twice every fortnight for 7–8 weeks). Windrows were turned using a Sandberger ST250 tractor-pulled compost turner (Sandberger GmBH Dittersdorf, Austria) and covered between turnings with TOPTEX Fleece (a gas permeable but water impermeable cover; Westcow Equipment Ltd. Wimbourne, UK) Household-waste compost (HWC) was obtained from a municipal recycling company (Newcastle Recycle, Morpeth, UK), which used a similar windrow-based composting system. Fresh *Brassica* tissue (FBT) was used to mimic and evaluate the effect of Brassica wastes. Organic brussels sprouts (*Brassica oleracea* var. *gemmifera*) were purchased in a supermarket and chopped into small (<1 cm pieces) using a food processor; brussels sprouts were used fresh immediately after purchase. All organic amendments were mixed with the soil using a concrete mixer. Substrates were then transferred into pots and watered to field capacity. Substrates were not covered with plastic sheets, a practice which has been used to optimise the biofumigation effect of Brassica soil amendments (Bailey and Lazarovits 2003; Matthiessen and Kirkegaard 2006). Tomato transplants were planted into soil within 2 days after organic matter amendments were applied to soil. Two different types of chitin were used: (a) Sigma practical grade (made from crab shells; Sigma C7170), and (b) a non-purified chitin product (made from crab and shrimp shells (Travena Organic Products Ltd., Newcastle, UK). Chitosan, a deacetylated chitin product (ChiPro, GmbH, Bremen, Germany), was added to the soil every week via the irrigation water. A commercial seaweed extract product ('Marinure', Glenside Organics Ltd. Stirling, UK) was applied with the irrigation water at the rate recommended by the manufacturers. A commercial *Bacillus subtilis* preparation was used, based on the *B. subtilis* strain MBI500 supplied by MicroBio Ltd. (St Albans, UK). Polyversum is a commercial product (Biopreparaty, Prague, Czech Republic) containing the BCA *P. oligandrum*. Both *B. subtilis* and Polyversum were applied at the concentration recommended by the manufacturers and added to pots twice: at planting and one month after planting. Apart from the treatments described above no other fertilisation or crop protection treatments were applied to soils.

A steam disinfection treatment was used as a positive control (Camplex HD5116 Electric Soil Steriliser, THERMOFORCE Ltd., Cumbria, UK). A fish emulsion-based liquid fertiliser (Nugro, Hortifeed, Lincoln, UK) was used as a fertilised-

Table 1 Summary of treatments, rates of application and NPK inputs associated with treatments used in experiments 1, 2 and 3

		Mineral nutrient inputs (g pot^{-1})[a]			Treatments used in experiment		
Treatment	Rate	N	P	K	1	2	3
Organic matter inputs							
CCM	50% (*v*/*v*)	125	40	118	X	X	X
	25% (*v*/*v*)	62	20	59		X	X
	12.5% (*v*/*v*)	31	10	29			X
HWC[b]	50% (*v*/*v*)	122	52	103	X		
FBT	25% (*v*/*v*)	9	1	7	X	X	X
	12.5% (*v*/*v*)	4	0.5	3		X	X
	6.25% (*v*/*v*)	2	0.3	2			X
Chitin (Sigma)	150 g pot^{-1}	12	0	0	X	X	
	75 g pot^{-1}	6	0	0		X	
Chitin (Travena)	150 g pot^{-1}	12	0	0		X	
	75 g pot^{-1}	6	0	0		X	
Chitosan	4.0 g pot^{-1} week^{-1}[c]	2.9	0	0		X	
	2.0 g pot^{-1} week^{-1}[c]	1.5	0	0		X	X
	1.0 g pot^{-1} week^{-1}[c]	0.7	0	0			X
	0.5 g pot^{-1} week^{-1}[c]	0.4	0	0			X
Seaweed extract	1 ml pot^{-1} week^{-1}[c]	0.1	0.1	1.1	X		
Control treatments							
Fish emulsion (fertilised negative control)	50 ml pot^{-1} week^{-1}[d]	55	22	55	X		
No treatment (non-fertilised negative control)		X	X	X	X	X	
Steam treatment (positive control)	Heat treatment at 81°C for 30 min				X	X	
Biological control treatments							
Bacillus subtilis	2 g pot^{-1} at planting and 1 month post-planting				X		
Pythium oligandrum (Polyversum)	2 g pot^{-1} at planting and 1 month post planting				X		
Combination treatments							
Chitosan+FBT	12.5% FBT, 1 g pot^{-1} week^{-1} chitosan[c]						X
Chitosan+CCM	12.5% CCM, 1 g pot^{-1} week^{-1} chitosan [c]						X
Chitosan+FBT+CCM	6.25% FBT + 6.25% CCM + 0.5 g pot^{-1} week^{-1} chitosan[c]						X

[a] Associated with inputs/treatments

[b] HWC was not included in experiments 2 and 3, because the composting facility used closed down and a source for HWC produced to a similar composting protocol could not be identified

[c] For the first 12 weeks only

[d] For the entire duration of the experiment

negative control treatment (see Table 1 for the mineral nutrients supplied by Nugro). It was applied with the irrigation water at a concentration recommended by the manufacturer for tomato crops that receive no additional organic matter-based fertility inputs. An unfertilised negative control was also included. Soil was watered to field capacity every 2 days.

Experimental design

All experiments were conducted in glasshouses managed by the School of Biology at Newcastle University. Experiments 1 and 2 were conducted in 2001 and 2002 between March and August respectively, while experiment 3 was conducted in 2003 between May and September. This resulted in differences in temperature profiles recorded for the three experiments (see Fig. 1a for the maximum and minimum daily temperature profiles during experiment 1 and 2 and Fig. 1b for the maximum and minimum daily temperature profiles during experiment 3). Individual pots were used as replicates, because in terms of soil pathogen inocula and amendments, they represented separate units/environments.

In experiment 1 each of the ten treatments was applied at only one rate to 12 pots with naturally corky root rot-infected soil. Pots were placed in a cool (to provide optimal conditions for corky root rot development) greenhouse in six blocks. A randomised complete block design (RCBD) was used and two pots (each with a single plant) of each soil treatment were included in each block. The individual treatments used in experiment 1 are listed in Table 1.

In experiment 2, soils infected with corky root rot only and a mixed pathogen inoculum (*P. lycopersicis* and *V. albo-atrum*) were used. To generate mixed soil

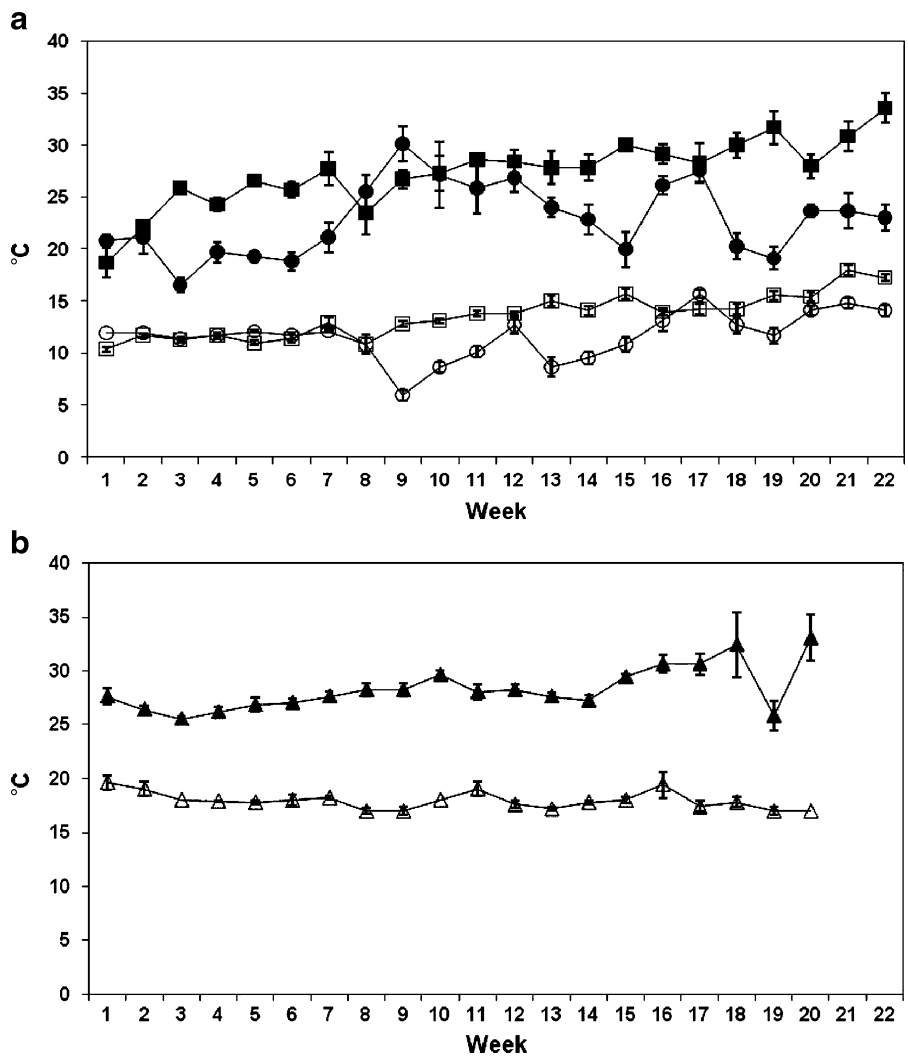

Fig. 1 Maximum (*filled symbols*) and minimum (*open symbols*) weekly temperatures in the glasshouses used in experiments. Graph **a**: experiments 1 (*circles*) and 2 (*squares*); graph **b**: experiment 3 (*triangles*)

inocula of corky root rot and *Verticillium*, soil naturally infected with corky root rot was artificially inoculated with *V. albo-atrum* strain AP01/036 (O'Neil 2002). Inoculum of this aggressive strain of *V. albo-atrum* was provided by Horticulture Research International (HRI, Wellesbourne, UK) as cultures grown on PDA (Oxoid). The root-injection method described by Sink and Grey (1999) was used for the infection of the rhizosphere of tomato seedlings: immediately before infection, mycelial plugs were taken from the PDA culture and used to prepare a conidial suspension using sterile distilled water (SDW). The suspension was then filtered/passed through a double layer of cheesecloth to remove mycelial fragments. The inoculum suspension was adjusted to 9×10^6 conidia ml^{-1} by dilution of the original suspension with SDW. Plants were not watered prior to inoculation to induce a slight drought stress and more rapid absorption of the spore inoculum suspension (Sink and Grey 1999). Each seedling was inoculated with two 5 ml injections of inoculum suspension into the rhizosphere.

Six pots each with a single plant were used for each of the 12 treatment×pathogen soil inoculum combinations included in the trial (=144 pots). Pots were placed in a cool greenhouse in six blocks. A RCBD was used and one pot of each treatment/pathogen inoculum combination was included in each block. The individual treatments included in experiment 2 are listed in Table 1.

In experiment 3, only the mixed pathogen inoculum was used. This was prepared as described for experiment 2. Five pots each with a single plant were used for each of the 13 individual or combination soil treatments included in the trial (=65 pots). Pots were placed in a cool greenhouse in five blocks. A RCBD was used and one pot of each treatment was included in each block. The individual and combination soil treatments are listed in Table 1. The objectives of experiment 3 were to identify **(a)** dose–response relationships of organic matter and chitin soil amendments and **(b)** the impact of combinations of soil amendments on crop performance; a soil steaming control treatment was therefore not included in these trials.

Assessments

Soil-borne disease symptoms were assessed at the end of the experiment. The roots of all plants were rinsed under tap water and root fresh weight (RFW) was determined. Plants were examined for visible corky root rot symptoms (see Pohronezny and Volin (1991) for a description of symptoms). The severity of corky root rot symptoms was not scored in experiments presented here. Instead, RFW was used as an indication of the level of root loss due to soil-borne diseases, since corky root rot severity is closely correlated with root loss (Last and Ebben 1966; Ebben 1974; Ebben et al. 1978; Pohronezny and Volin 1991). The difference in RFW between plants grown in steam-disinfected and non-steam-disinfected soils was used as a measure of overall root damage/loss caused by the different soil-borne pathogen inocula. In experiments 2 and 3, root dry weights were also determined, but since differences between treatments followed the same trends as those found for RFW, they are not shown here.

For yield assessments, tomato fruits were collected at 'uniform red' stage (according to colour charts used in commercial glasshouse production) twice per week. New shoots developing on the main stem were removed and all trusses bearing fruit developing on the main stem were included in yield assessments. Fruit number, weight and diameter were also recorded.

Dehydrogenase activity (DH) was used as a measure of SBA (Ross 1971; Mersi and Schinner 1991). Dehydrogenase activity was determined based on soil treatment with iodonitrotetrazolium chloride using the method described by Mersi and Schinner (1991). DH is expressed as microgram of iodonitrotetrazolium formazan (INTF) produced g^{-1} dry soil within a 2 h period (μg INTF g^{-1} 2 h^{-1}) after addition of iodonitrotetrazolium chloride to soil.

Statistical analyses

Results were analysed with a general linear model in Minitab and the residuals were tested for normality with the Anderson–Darling test. Where the residuals were significantly different to a normal distribution and could not be transformed satisfactorily, a non-parametric test (Mann–Whitney) was used. Where significant differences were found in the GLM Tukey's Honest Significant Difference tests were used to compare individual means and the lettering convention used in tables to indicate means that are significantly different. In addition, the relationships

between some measurements were investigated using Pearson's product-moment correlations.

In the analysis of data from experiment 2, which tested for interactions between pathogen inocula (*P. lycopersici* alone or *P. lycopersici* plus *V. albo-atrum*) and different soil amendment treatments with respect to tomato crop performance, results obtained for plants grown in steam-disinfected soil were not included. This was done because the steam sterilisation (at >80°C) method used was previously shown to completely remove fungal pathogen inocula from soil (Van Loehnen et al. 2003) and inclusion of the data for plants grown in steam-disinfected soil would have masked the differences between pathogen inoculum treatments in the statistical tests. However, Dunnett's tests were used to compare individual interaction means with the steamed and untreated controls. Where the pathogen level was shown to interact with the other factors in the analysis, separate analyses were carried out for the data sets obtained with the two different soil inocula (*P. lycopersici* alone or *P. lycopersici* plus *V. albo-atrum*) and in these analyses data from both untreated and steam-disinfected soils were included. In this analysis means were compared using Tukey's HSD test.

Results

Effect of soil amendments on root disease and fruit yield (experiment 1)

No visible corky root rot symptoms were found in roots of plants grown in steam-disinfected control soils. Five of the nine soil treatments resulted in a significantly increased yield (Table 2) compared to plants grown in untreated soil. This included plants grown in soil treated with **(a)** steam (positive control), **(b)** CCM, **(c)** HWC, **(d)** FBT or **(e)** chitin (Sigma). There were no significant differences in yield among these treatments. The same soil treatments (with the exception of the CCM treatment) also resulted in a significantly higher RFW compared to plants grown in untreated soil (Table 2).

The two BCA treatments (*P. oligandrum* and *B. subtilis*), the seaweed extract and the fish emulsion treatment did not significantly affect yield or RFW when compared to plants grown in untreated soil (Table 2).

HWC, FBT and chitin treatment also significantly increased fruit number per plant compared to plants grown in untreated soil, but none of the treatments had a significant effect on fruit diameter, except for CCM which significantly increased fruit diameter compared to the fish emulsion treatment (=fertilised negative control) (Table 2).

Effect of (and interactions between) soil-borne pathogen inocula, soil amendment type and input level on root disease and fruit yield (experiment 2)

In experiment 2, the total fruit yield per plant was highest when the soil was steamed independent of soil infestation with corky root rot only or both corky root rot and *Verticillium* (Table 3). Only the individual treatment combinations of chitosan at either the low or high rate resulted in yields equivalent to the steamed treatment (based on results of Dunnett's test at $P<0.05$). When the main effects of amendment type were compared, both chitosan and Sigma chitin treatments had the highest yields, regardless of the level of amendment (Table 3). The higher level of amendment resulted on average in higher total fruit yields, and there were no significant interactions among the main effects.

Numbers of fruit per plant were only affected by the level of amendment, with significantly more fruit harvested per plant at the higher rate of amendment. In contrast, mean fruit diameter was not affected by the level of amendment or the level of pathogen inoculum, but was affected by amendment type. Amendment with chitosan resulted in the largest mean fruit diameters while CCM, FBT and Travena chitin had the smallest fruit diameters.

Overall, RFWs were highest when chitosan was used as an amendment (Table 3). This was the case regardless of the rate of chitosan application and the type of pathogen present in the soil (Table 4). None of the soil amendments resulted in RFW as high as the soil steaming treatment (Table 4). There were significant interactions between the pathogen inoculum type and the level of soil amendment ($P=0.003$) and between the amendment type and the level of that amendment ($P=0.025$). When only corky root rot-infected soil was used, the level of the amendment did not affect the RFW; however, when soils were

Table 2 Effect of soil treatments (refer to Table 1 for the input levels used) on tomato yield parameters (experiment 1)

Soil treatment	Total fruit yield (kg plant^{-1})	RFW (g plant^{-1})^{L}	Number of fruit per plant	Fruit diam (cm)
Steaming (positive control)	1.3 a (0.3)	81 a (14)	34 a (6)	4.2 ab (0.2)
Untreated (negative control)	0.7 b (0.1)	45 c (11)	25 b (4)	4.0 ab (0.7)
CCM 50% (*v*/*v*)	1.2 a (0.3)	54 bc (15)	30 ab (5)	4.4 a (0.5)
HWC 50% (*v*/*v*)	1.2 a (0.3)	64 ab (19)	35 a (6)	4.0 ab (0.3)
FBT 25% (*v*/*v*)	1.2 a (0.5)	80 a (33)	37 a (6)	4.2 ab (0.5)
Chitin (Sigma) 1% (*w*/*v*)	1.2 a (0.2)	81 a (21)	35 a (7)	4.0 ab (0.2)
Seaweed extract	0.7 b (0.2)	45 c (9)	22 b (6)	4.0 ab (0.5)
Fish emulsion (fertilised negative control)	0.8 b (0.1)	48 c (7)	26 b (4)	3.7 b (0.3)
B. subtillis	0.7 b (0.2)	49c (13)	23 b (5)	3.8 ab (0.3)
P. oligandrum	0.7 b (0.2)	46 c (10)	23 b (5)	3.7 b (0.3)

One way ANOVA showed that there were significant differences between treatments ($P<0.001$ for total fruit yield and RFW; $P<0.01$ for number of fruit and fruit diameter). Means within the same column followed by the same letter are not significantly different according to Tukey's Honest Significant Difference Test ($P<0.05$). Figures in brackets are standard deviations of each mean

infected with both corky root rot and *Verticillium*, in some cases the level of amendment affected RFW. For Sigma chitin and Travena chitin a higher level of amendment resulted in a significant increase in root yield in soils infected with both corky root rot and *Verticillium*. In contrast, for FBT, CCM and chitosan, doubling the level of amendment did not result in a significant increase in RFW.

Table 3 Main effect means and ANOVA *P*-values for tomato production parameters (experiment 2)

Main effect means	Total fruit yield (kg plant^{-1})	Number of fruit per plant	Fruit diam (cm)	RFW (g)
Soil steaming[a]	1.1 (0.2)	23 (4)	4.7 (0.3)	71 (9)
Untreated[a]	0.4 (0.1)	12 (2)	4.1 (0.2)	16 (3)
Pathogen type (P)				
P. lycopersici	0.82 (0.2)	19 (4)	4.3 (0.3)	37 (13)
P. lycopersici+*V. albo-atrum*	0.75 (0.3)	19 (4)	4.3 (0.3)	34 (17)
Amendment type (A)[b]				
FBT	0.79 ab (0.1)	21 (3)	4.3 b (0.2)	30 c (4)
CCM	0.66 b (0.1)	17 (3)	4.2 b (0.3)	29 c (4)
Chitin (Sigma)	0.87 a (0.2)	20 (3)	4.4 ab (0.2)	43 b (9)
Chitin (Travena)	0.71 b (0.2)	18 (4)	4.3 b (0.3)	28 c (6)
Chitosan	0.91 a (0.2)	20 (4)	4.5 a (0.3)	48 a (7)
Amendment level (L)				
Low	0.75 (0.2)	18 (4)	4.3 (0.3)	34 (10)
High	0.82 (0.2)	20 (3)	4.3 (0.3)	37 (10)
ANOVA *P*-values				
P	ns	ns	ns	0.003
A	0.001	ns	0.012	<0.001
L	0.037	0.008	ns	0.001
P×*A*	ns	ns	ns	ns
P×*L*	ns	ns	ns	0.003
A×*L*	ns	ns	ns	0.025
P×*A*×*L*	ns	ns	0.031	ns

Figures in brackets are standard deviations of each mean

[a] Means for these treatments presented for comparison purposes (not included in the ANOVA)

[b] Means for each amendment type in the same column followed by the same letter are not significantly different (ns) at the $P=0.05$ significance level (Tukeys HSD)

Table 4 Effect of soil amendment on RFW for soil inoculated with *P. lycopersici* alone, and *P. lycopersici* plus *V. albo-atrum*[z] (experiment 2)

Soil amendment/ treatment	RFW (g)	
	P. lycopersici	*P. lycopersici* plus *V. albo-atrum*
Soil steaming	64 a (4)	77 a (8)
Untreated	18 d (1)	13 e (2)
FBT (25.0%)	31 c (4)	28 c (5)
FBT (12.5%)	31 c (4)	28 c (3)
CCM (50%)	29 c (5)	29 c (5)
CCM (25%)	29 c (4)	28 c (5)
Chitin (Sigma) (1.0%)	47 b (8)	47 b (6)
Chitin (Sigma) (0.5%)	45 b (7)	30 c (3)
Chitin (Travena) (1.0%)	31 c (4)	30 c (4)
Chitin (Travena) (0.5%)	29 c (6)	19 d (2)
Chitosan (4 g plant^{-1})	47 b (9)	48 b (6)
Chitosan (2 g plant^{-1})	48 b (7)	47 b (6)

Means followed by the same letter in the same column are not significantly different at the $P=0.05$ significance level (Tukeys HSD). Figures in brackets are standard deviations of each mean

Effect of different levels of chitin and organic matter amendments on soil biological activity, root disease and fruit yield (experiment 3)

Since experiment 2 had indicated that a reduction in the level of soil amendments will increase soil-borne disease (i.e. reduce RFW), a wider range of soil amendment input levels were tested for their activity against soil-borne diseases (only soils infected with both corky root rot and *V. albo-atrum* were used). Also, DH was measured in soils from all treatments, to identify whether an increase in SBA was associated with the use of soil amendments, thus indicating a potential mechanism for the reduction in soil-borne disease levels observed.

There was no significant increase in SBA with increasing levels of chitosan addition; however, RFW and fruit yield, diameter and number increased with increasing rate of chitosan amendment (Table 5), although the difference between the two higher chitin input levels was not statistically significant. There was a significant correlation between RFW and yield ($r=0.86$; $P<0.001$), but no significant correlation between SBA and either RFW or fruit yield.

For FBT there was a significant increase in SBA, RFW and fruit yield, diameter and number with increasing levels of FBT addition to soil (Table 5). However, the difference between the two higher FBT input levels was only statistically significant for fruit yield. Also, the difference between untreated and the lowest level of FBT was only significant for fruit yield (Table 5). There were significant correlations between SBA and both **(**a**)** RFW ($r=0.54$; $P=0.001$) and (b**)** fruit yield ($r=0.51$;

Table 5 Effect of level of chitosan, FBT and CCM on SBA, RFW, fruit diameter and number of fruit per plant; experiment 3

Amendment type (level)	DH (μg INTF×g^{-1} DM×2 h^{-1})	RFW (g plant^{-1})	Fruit yield (kg plant^{-1})	Fruit diam (cm)	Number of fruit per plant
Untreated (negative control)					
0	62 b (21)	34 d (8)	0.8 d (0.1)	3.2 c (1.0)	28 e (3)
Chitosan (g pot^{-1} week^{-1})					
0.5	58 b (6)	51 bc (4)	1.2 c (0.2)	3.4 bc (2.3)	39 de (7)
1.0	63 b (12)	70 a (7)	1.6 ab (0.1)	3.6 ab (1.0)	54 ab (7)
2.0	64 b (13)	70 a (8)	1.8 a (0.1)	3.9 a (1.4)	67 a (8)
FBT (% pot^{-1} *v*/*v*)					
6.25	57 b (10)	49 cd (5)	1.2 c (0.1)	3.5 bc (1.7)	38 de (5)
12.5	78 ab (9)	60 abc (5)	1.2 c (0.1)	3.5 bc (1.4)	40 cde (8)
25	98 a (19)	65 ab (9)	1.5 b (0.1)	3.7 ab (1.8)	53 bc (11)
CCM (% pot^{-1} *v*/*v*)					
12.5	91 a (8)	58 ab (5)	1.2 c (0.1)	3.6 ab (0.8)	40 cde (7)
25	101 a (15)	61 a (8)	1.4 bc (0.1)	3.7 ab (1.0)	46 bcd (7)

Not all treatments included in experiment 3 are shown here, but ANOVA and Tukey's. Honest Significant Difference Test are from an analysis including all treatments. One-way ANOVA showed significant ($P<0.001$) differences between treatments for all parameters assessed. Means within columns with the same letters are not significantly different according to Tukey's Honest Significant Difference Test ($P<0.05$). Figures in brackets are standard deviations of each mean

P=0.020), and a highly significant correlation between RFW and yield (r=0.78; P<0.0001).

CCM increased SBA, RFW and fruit yield, diameter and number when compared to the untreated control (Table 5); however, the difference between the two CCM input levels was not significant (Table 5). There were significant correlations between SBA and both **(a)** RFW (r=0.54; P=0.035) and **(b)** fruit yield (r=0.73 P=0.002), and a very highly significant correlation between RFW and yield (r=0.76; P=0.001).

Effect of using combinations of soil amendments on SBA, root disease and fruit yield (experiment 3)

There were virtually no additive effects when treatment combinations of (a) chitosan (1 g plant^{-1} week^{-1}) and CCM (12.5% *v*/*v*), (b) chitosan (0.5 g plant^{-1} week^{-1}), FBT (6.25% *v*/*v*) and CCM (6.25% *v*/*v*) were used and (c) chitosan (at 1 g plant^{-1} week^{-1}) and FBT (at 12.5% *v*/*v*) were compared to the component treatments applied at the same and twice the input level used in the combination treatment (data not shown). However, for the number of fruit an additive effect between chitosan (at 1 g plant^{-1} week^{-1}) and FBT (at 12.5% *v*/*v*) could be detected (data not shown).

Discussion

Efficacy of soil amendments as alternative to chemical and thermal soil disinfection

Two of the organic matter amendment-based treatments (FBT and CCM) reduced disease and increased yields significantly in comparison to the untreated control in all three experiments at all input levels. The same treatments and HWC were also found to give similar levels of control against soil-borne diseases to steam disinfection in experiment 1. Organic matter inputs at levels that provided significant levels of disease control (25% to 50% *v*/*v*) are commonly used in organic production systems, which omit or minimise the use of allowable water-soluble NPK fertilisers (e.g. products made from fish slurries, spent fungal growth media or chicken manure) (Soil Association 2005).

A major problem when introducing household waste-based composts is the lack of uniformity between batches of compost and it is therefore important to develop quality assurance protocols that guarantee reproducible disease suppression and nutrient release characteristics. Similarly, to translate the results obtained with brussels sprout tissue into commercially practical control methods e.g. methods based on soil incorporation of *Brassica* processing waste and/or the introduction of *Brassica* crops into soil-based, protected tomato production systems requires detailed knowledge about the glucosinolate profiles and associated biofumigation potential of *Brassica* spp. used as waste or break crops (Kirkegaard and Sawar 1998). Also, the cost of available waste composts, fresh *Brassica* waste and/or the introduction of *Brassica* break crops in glasshouse production needs to be considered. However, both household waste-based composts and certain *Brassica* break crops (e.g. Kohlrabi) are now used in some organic glasshouse tomato production systems in Europe (Cantelo Nurseries Ltd., personal communication).

Many organic greenhouse systems use much lower organic matter input levels and supply up to 50% of NPK in the form of permitted water soluble NPK fertilisers (Soil Association, personal communication). Many of these fertilisers are expensive and have raised concerns with respect to their sustainability (fish slurry-based products are transported between continents and their use may contribute to unsustainable fishing practices). Replacing permitted water-soluble NPK fertilisers with disease-suppressive manure or communal/household waste-based composts in organic farming systems would therefore not only minimise the need for soil disinfection treatments, but also increase the sustainability of such systems.

Chitin/chitosan treatments also provided high levels of control against both corky root rot and *V. albo-atrum* and can therefore also be recommended as an alternative to soil disinfection treatments. The use of chitin (but also FBT-based soil amendments) may be of particular interest in areas where limited amounts of manure or composted communal/household waste-based fertility inputs are available.

Organic matter or chitin soil amendments can also form part of integrated crop protection systems aiming to replace the soil disinfectant methyl bromide

in conventional, soil-based greenhouse production systems. It may be particularly useful where *Verticillium* spp. are a major problem. Munnecke et al. (1978) categorised *V. albo-atrum* as the pathogen least sensitive to methyl bromide, when compared to several other soil-borne plant pathogenic fungi including *Pyrenocheta lycopersici*. Moreover, Bourbos (1986) demonstrated that 12 days after treatment with methyl bromide in unheated tomato glasshouses, *V. albo-atrum* started to re-appear, and that the treatment did not affect fungi at a depth of 30–40 cm; *Verticillium* inocula present at such depths can re-colonise upper layers of soil within one growing period. This is thought to be the main reason for the very frequent need to re-apply methyl bromide treatments in *Verticillium*-infested soils. Soil fumigation with chloropicrin, isothiocyanates and other chemicals (Campbell et al. 1982; Wambeke et al. 1984; Overman and Jones 1986) can be effective in controlling soil-borne diseases but, compared to methyl bromide, most other chemical soil disinfectants are either less effective or currently uneconomic and are therefore used for only a small proportion of glasshouse production (Wambeke et al. 1984; Jones et al. 1987).

Link between soil biological activity, soil-borne disease levels and crop yield

The significant correlations between the SBA and the severity of soil-borne disease severity (assessed as RFW) when organic matter soil amendments (FBT and CCM) were used, indicate that increased competition from the saprophytic soil biota associated with the increased SBA may have been a mechanism for the reduced incidence of root disease (i.e. higher RFWs and RDWs) and higher fruit yields, size and numbers recorded.

This result has confirmed earlier studies which showed that the incorporation of organic matter into soils increases soil microbial activity and/or levels of antagonistic microorganisms in soil (Xiao et al. 1998). However, release of antimicrobials from Brassica tissues may also have contributed to the observed reduction in root disease and improvement in crop performance (Sarwar and Kirkegaard 1998; Kirkegaard and Sawar 1998). Previous studies showed that the suppression of soil-borne diseases associated with the inclusion of Brassica crops in arable crop rotations was linked to release of biocidal compounds (especially isothiocyanates) when glucosinolates present in Brassica tissues are hydrolysed during the decomposition in soil (Morra and Kirkegaard 2002; Bailey and Lazarovits 2003; Matthiessen and Kirkegaard 2006). Whether the same mechanism was responsible for the suppression of soil-borne diseases of tomato reported here will have to be determined in future studies.

The supply of additional nutrients associated with mineralisation of organic matter inputs may also have contributed to the observed increases in fruit yield, size and number, since it may have improved the plant's ability to compensate for root losses caused by soil-borne pathogens. An untreated-fertilised control was therefore included in experiment 1. The fish emulsion fertiliser used contained N in a readily plant available form and was applied at a level equivalent to 50% of total N applied with the highest organic matter-based inputs (manure and household waste compost). Since it is known that only approximately 50% of N present in organic fertilisers is mineralised and becomes plant available in the first 12 months after application (Finck 1979) the fish emulsion fertiliser was estimated to provide approximately the same nutrient supply as the highest organic matter-based amendment used in the experiment. Since untreated non-fertilised and fertilised plants showed similarly high levels of disease and fruit yield, size and numbers it was assumed that improvements in performance associated with other treatments were mainly due to suppressive effects. However, for some treatments (e.g. CCM) it is likely that increased nutrient supply has to some extent compensated for root losses caused by soil-borne diseases. This should be investigated further in future studies.

Since chitosan treatment did not increase SBA it is unlikely that increased competition from the soil biota has contributed to the reduction in root disease (i.e. higher RFWs and RDWs) and increased fruit yield, size and number associated with chitosan treatments of soils. However, previous studies have suggested a range of other mechanisms of action for chitin and/or chitosan treatment of plants. For example, chitosan has been reported to act as an elicitor and induce resistance against root diseases (Reddy et al. 1999). Other studies suggested that the growth promotion could be caused by the additional nitrogen supplied by chitin treatment (chitosan contains 8.7% N) (Ohta

et al. 1999). Since chitosan was shown to form thin films around soil structures it was also hypothesised that chitosan may act as a physical barrier preventing the invasion of soil pathogens and/or exudation of nutrients from roots to an extent that reduces the ability of the pathogens to attack plant roots (El Ghaouth et al. 2000). However, the exact mode of action of chitin/chitosan treatments will have to be determined in future studies.

It is also important to note that the temperature profiles for experiments 1 and 2 (where SBA was not measured) differed considerably from those in experiment 3 (where SBA was measured), which was carried out during a warmer period of the year. This is likely to have been a confounding factor, since both disease severity of corky root rot and SBA are known to decrease with increasing soil temperature (Last and Ebben 1966; Grove and Campbell 1987; Mersi and Schinner 1991; Workneh and Van Bruggen 1994; Bailey and Lazarovits 2003). Future studies should therefore investigate the impact of different soil amendments under contrasting environmental conditions (including soil temperature, matric potential and N-status) to identify more precisely the 'window of opportunity' for different soil amendments.

Potential economic impacts

Apart from having significant negative effects on SBA and structural stability, steam and methyl bromide also represent a significant variable cost (costing approximately 2,500 to 4,500 € ha^{-1}, depending on the system used; Cantelo Nurseries Ltd., personal communication). The potential to replace steam and chemical soil disinfection with the soil amendment-based protocols shown in this study could therefore provide both environmental and economic benefits for farmers if the costs of alternative treatments do not exceed those of currently used soil disinfection protocols.

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