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Effect of inoculum rates and sources of *Coniothyrium minitans* on control of *Sclerotinia sclerotiorum* disease in glasshouse lettuce

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Abstract

Coniothyrium minitans isolate Conio grew on both maizemeal-perlite and ground maizemeal-perlite, producing high numbers (1.6×10^7 conidia g^{-1} inoculum) of germinable conidia. *Coniothyrium minitans* isolate Conio applied as a preplanting soil incorporation of maizemeal-perlite inoculum at full application rate (0.6 l m^{-2}; 10^{11} colony forming units (cfu) m^{-2}) significantly reduced Sclerotinia disease in a sequence of three lettuce crops grown in a glasshouse. No reduction in disease was achieved with any of the reduced rate treatments (10^8 cfu m^{-2}) of a range of *C. minitans* isolates (Conio ground maizemeal-perlite at reduced rate, Conio and IVT1 spore suspensions derived from maizemeal-perlite, IVT1 spore suspension derived from oats and Contans® WG spore suspension). After harvest of the second and third crops, *C. minitans* maizemeal-perlite at full rate reduced the number and viability of sclerotia recovered on the soil surface and increased infection by *C. minitans* compared with spore suspension and reduced rate maizemeal-perlite inocula. *Coniothyrium minitans* was recovered from the soil throughout the trial, between 10^5 and 10^7 cfu cm^{-3} in maizemeal-perlite inoculum full rate treated plots and 10^1–10^4 cfu cm^{-3} in all other inoculum treated plots.

Pot bioassays were set up corresponding to the inoculum used in the glasshouse, with the addition of Conio ground maizemeal-perlite at a rate corresponding to the full rate maizemeal-perlite. *Coniothyrium minitans* maizemeal-perlite and ground maizemeal-perlite at full rate significantly decreased carpogenic germination, recovery and viability of sclerotia and increased infection of sclerotia by *C. minitans* in comparison with spore suspension treatments, reflecting results of the glasshouse trials. Additionally, reduced maizemeal-perlite treatment also decreased apothecial production, recovery and viability of sclerotia compared with the spore suspension treatment, despite being applied at similar rates. Simultaneous infection of sclerotia by several isolates of *C. minitans* was demonstrated. Inoculum level in terms of colony forming units cm^{-3} of soil appears to be a key factor in both control of Sclerotinia disease and in reducing apothecial production by sclerotia.

Introduction

Sclerotinia sclerotiorum is a major plant pathogen of several crops world-wide including glasshouse grown lettuce (Boland and Hall, 1994). Initiation of disease in glasshouse grown lettuce is generally from ascospores originating from outside the glasshouse, with disease spreading through plant to plant contact. Sclerotia that are formed on the diseased plants return to the soil enabling the pathogen to survive between crops (Coley-Smith and Cooke, 1971; Merriman, 1976). These sclerotia germinate to produce a mycelium which infects plants directly, or more typically in glasshouse lettuce in the UK, they germinate carpogenically to produce apothecia, releasing ascospores which can infect aerial plant parts. Prophylactic spraying with fungicides can prevent ascospore infection and hence reduce crop losses. However, disease can still become established due to problems in achieving effective spray penetration of the crop canopy, and also

the need in the UK for a 4-week interval for winter crops between the final fungicide spray and harvest. When disease builds up in glasshouse soil, sclerotia can be eliminated by steam sterilisation or fumigation with chemicals such as methyl bromide. However, the future withdrawal of methyl bromide and the expense of steam sterilisation, coupled with environmental concerns over the regular use of fungicide sprays and the decreasing number of effective fungicides, has prompted an increased interest in the biological control of *S. sclerotiorum*.

The sclerotial parasite *C. minitans* has been shown to infect and degrade sclerotia in soil (Tribe, 1957; Huang, 1980; Trutmann et al., 1980; Whipps and Budge, 1990; McQuilken and Whipps, 1995) and to have potential for biocontrol of *S. sclerotiorum*. Several solid-substrate inocula of *C. minitans* applied to soil prior to planting, have been shown to control *S. sclerotiorum* at low disease levels in both glasshouse and field crops (Huang, 1980; Lynch and Ebben, 1986; Whipps et al., 1989; 1993; Budge and Whipps, 1991; McLaren et al., 1994; McQuilken and Whipps, 1995; Budge et al., 1995). Integration with a single foliar application of iprodione has also demonstrated that integrated control is possible (Budge and Whipps, 2001). There was little difference in the efficacy of five solid-substrate inocula applied as single preplanting soil incorporation at a standard rate of $0.6\,l\,m^{-2}$ (McQuilken and Whipps, 1995) and increasing the application rate of maizemeal-perlite solid-substrate inoculum fivefold did not have any additional disease control effect (Budge et al., 1995). This indicated that the standard application rate used was in excess of the optimum rate for disease control. Significantly, it has been argued that incorporation of large volumes of solid-substrate inocula might be uneconomical for commercial use, with spore incorporation being a more realistic approach (Whipps and Gerlagh, 1992; Jones and Stewart, 2000) and *C. minitans* spore sprays applied to diseased plants or crop debris have been shown to decrease Sclerotinia disease in subsequent crops (Verdam et al., 1993; Evenhuis et al., 1995; Budge et al., 1995; Gerlagh et al., 1999; Huang et al., 2000). However, spore suspensions as preplanting soil incorporations have not been tested.

In this study, the effect of different application rates of maizemeal-perlite inoculum on control of Sclerotinia disease in a sequence of three glasshouse lettuce crops was investigated. In addition, soil incorporation of spore suspension inocula of two *C. minitans* isolates and a spore suspension of a commercial *C. minitans* biocontrol product, Contans® WG, were also tested. To complement the glasshouse lettuce trial, glasshouse pot bioassays were conducted to investigate the effect of the inocula on apothecial production, sclerotial viability and infection of recovered sclerotia by *C. minitans*.

Materials and methods

Source and maintenance of fungi

Two isolates of *C. minitans* were used: IVT1 (supplied by Dr M. Gerlagh) and Conio (IMI 134523). *Sclerotinia sclerotiorum* originally isolated from glasshouse lettuce (SB; Whipps and Budge, 1990; McQuilken and Whipps, 1995) was used. Both fungi were stored in polypropylene straw ampoules in liquid N_2 (Challen and Elliott, 1986) and routinely cultured at 18–20 °C on potato dextrose agar (PDA; Oxoid, UK).

To maintain the pathogenicity of the *S. sclerotiorum* isolate used, lettuce (*Lactuca sativa* L. cv. Rachel) were artificially inoculated with *S. sclerotiorum* by placing agar discs of the fungus onto injured leaf petioles. The disease was allowed to develop, and the sclerotia produced, harvested and air-dried. These were surface sterilised (Williams et al., 1998a) and placed on PDA to obtain a fresh culture.

Production of Sclerotinia sclerotiorum *sclerotia*

The large quantities of sclerotia required for the pot bioassays were produced on sterilised wheat grain (cv. Armada). Flasks containing 50 ml de-ionised water and 25 g wheat grain were autoclaved (0.1 MPa, 122 °C, 15 min), allowed to cool, and inoculated with two agar plugs (5 mm dia.) taken from the margin of an actively growing PDA culture of *S. sclerotiorum* (Mylchreest and Wheeler, 1987). After incubation at 18–20 °C for 4–6 weeks, the flasks were incubated at 4 °C for a further 4 weeks. The sclerotia were washed, dried overnight in a stream of sterile air and those of 2–4 mm dia. were selected and used immediately.

Production of solid-substrate inocula of Coniothyrium minitans

Maizemeal-perlite and ground maizemeal-perlite were tested as solid substrates for inoculum production of *C. minitans*. Ground maizemeal-perlite was used to prepare reduced rate maizemeal-perlite inocula for the

glasshouse trial and the pot bioassays. Maizemeal-perlite was prepared using a modification of the method developed by McQuilken and Whipps (1995). Two-litre quantities of a 15% v/v mixture of micronised flaked maize (Midland Shires Farmers Ltd., Worcester, UK) and horticultural grade perlite (Silvaperl Products Ltd., Harrogate, UK), were mixed with 400 ml tap water, and placed in spawn bags ($22.5 \times 56\,cm^{-2}$; Van Leer Ltd., Poole, UK) and autoclaved (0.1 MPa, 122 °C, 15 min).

Ground maizemeal-perlite inocula were prepared using a similar method, whereby 2 litre quantities of a 15% v/v mixture of micronised flaked maize and horticultural grade perlite were ground using a Cyclotec 1093 mill (Perstorp Ltd., Maidenhead, UK) to <1 mm particle size. This was then mixed with 300 ml tap water and autoclaved (0.1 MPa, 122 °C, 15 min) in spawn bags.

Three replicate bags of each solid substrate were inoculated with 200 ml of a 1×10^6 spores ml^{-1} spore suspension produced from 14-day-old PDA cultures of both *C. minitans* isolate Conio and IVT1. Bags were incubated at 18–20 °C for 28 days, with the bags shaken every week to distribute the growing mycelia. To assess conidial production, three replicate 1 g samples of inocula from each bag were macerated individually in 100 ml sterile 0.01% (w/v) Oxoid agar using a laboratory mixer/emulsifier (Silverson Machines Ltd. Chesham, Bucks, UK) operated at full speed for 2 min. Spore suspensions were then passed through 2 layers of muslin to remove debris and the number of conidia counted using a haemacytometer (McQuilken and Whipps, 1995). Colony forming unit (cfu) counts of the inocula were assessed by plating serial dilutions of the spore suspensions onto PDA containing Triton X-100 ($2\,ml\,l^{-1}$; Sigma Chemicals, USA) and chlortetracycline ($20\,mg\,l^{-1}$ powder containing 80% chlortetracycline HCl, Sigma Chemicals, USA). These were incubated at 18–20 °C for 14 days, after which colonies of *C. minitans* were counted and cfu g^{-1} of inoculum calculated (McQuilken and Whipps, 1995). Conidial germinability was assessed by concentrating spore suspensions by centrifugation at $9000 \times g$ for 5 min and resuspending in sterile de-ionised water to give a final concentration of 1×10^6 spores ml^{-1}. Aliquots (0.5 ml) of spore suspension were spread over the surface of four PDA plates containing chlortetracycline ($20\,mg\,l^{-1}$), and the dishes were placed in sealed plastic bags and incubated at 18 °C in the dark for 36 h. The plates were then flooded with lactophenol aniline blue and germination of four counts of 100 conidia on each plate were scored. A conidium was considered to have germinated when the germ tube length was equal to or greater than the length of the spore.

Production of spore suspension inocula of Coniothyrium minitans

Spore suspensions of Conio or IVT1 were prepared from the maizemeal-perlite inocula by adding 2 litre tap water to each bag, agitating the contents for 1 min, then passing the supernatant through a 180-μm sieve. Microscopic examination of the resulting filtrate revealed that no pycnidia and little debris passed through the sieve. The concentration of spore suspensions produced in this way was assessed using a haemacytometer. Suspensions were diluted with tap water as required for application.

IVT1 was also grown on oat grains by large-scale solid-state fermentation (Durand et al., 1994) and this inoculum was supplied by Dr A. Durand and N. Antoine INRA, Dijon, France. Spore suspensions were prepared as described above for maizemeal-perlite inocula, whereby, 2 litre of the oat-based inoculum was placed in a spawn bag, and to this 2 litre of tap water was added and the bag agitated for 1 min. The resulting supernatant was treated as for maizemeal-perlite spore suspension inocula.

Spore suspensions of Contans® WG (granular material containing 1×10^9 conidia g^{-1}; Prophyta GmbH, Malchow/Poel, Germany) were prepared by adding 0.1 g dry material to 1 litre tap water and stirring for 30 min. Appropriate dilutions for application were then prepared on the basis of haemacytometer counts.

Glasshouse trial

The effect of a range of different forms and rates of inocula of *C. minitans* on disease caused by *S. sclerotiorum* was assessed in a series of sequential lettuce crops grown in the glasshouse.

Disease establishment

Lettuce (*Lactuca sativa* L. cv. Rachel) was planted throughout a Wilco glasshouse chamber ($30 \times 6\,m^{-2}$; Wilco Horticultural Ltd., Hull, UK) and artificially inoculated with *S. sclerotiorum* isolate SB to produce a natural population of sclerotia in the soil (Budge et al.,

1995). Plants were watered by overhead irrigation as required. At harvest (2 July 1998), healthy plants were removed and diseased crop debris left *in situ*. When dry, the debris together with newly-formed sclerotia were evenly-spread throughout the chamber and the soil was rotovated to a depth of 15 cm.

Experimental design

The chamber was marked out into 24 plots ($1.4 \times 2.6\,m^2$) separated by 0.4 m paths to give 3 plots across and 8 plots along the east-west orientated chamber. The number of sclerotia on the soil surface in three randomly-positioned quadrats (500 cm^2 each) in each plot was counted.

Eight treatments were applied to separate plots: (i) control: no treatment; (ii) control: fungicide spray (iprodione); (iii) Conio maizemeal-perlite soil incorporation; (iv) Conio ground maizemeal-perlite soil incorporation at a reduced rate corresponding to that applied with spore suspension inocula; (v) Conio spore suspension soil incorporation, produced from maizemeal-perlite inocula; (vi) IVT1 spore suspension soil incorporation, produced from maizemeal-perlite inocula; (vii) IVT1 spore suspension soil incorporation, produced from oat inocula; and (viii) Contans® WG spore suspension soil incorporation. The treatments were allocated to plots within the 8 by 3 array following an incomplete row and column design, with a complete replicate set of treatments occurring in each row of 8 plots, and pairs of treatments only occurring together within a column of 3 plots at most once. Three sequential lettuce crops were planted, the first on 5th August 1998 and harvested 6 weeks later, the second on 20th October 1998 and harvested 16 weeks later and the third crop on 26th February 1999 and harvested 10 weeks later, providing inter crop periods of 5 and 2 weeks, respectively.

Immediately before planting each lettuce crop, 2.2 litre of maizemeal-perlite inoculum of Conio was evenly applied to each appropriate plot (0.6 litre inoculum m^{-2}) and raked into the soil surface to a depth of about 3 cm. Inocula of Conio for the first, second and third crop, respectively consisted of 1.7×10^8, 1.3×10^8 and 1.8×10^8 cfu cm^{-3} maizemeal-perlite. This gave 1.0×10^{11}, 7.7×10^{10} and 1.1×10^{11} cfu m^{-2} soil for the first, second and third crop, respectively, and was recovered at 1.0×10^6, 0.8×10^6 and 0.4×10^6 cfu cm^{-3} soil after the first, second and third application. For the reduced rate ground maizemeal-perlite inoculum of Conio, the amount of ground maizemeal-perlite inocula required to give the same inoculation rate as for spore suspension was calculated (7.4 ml per plot). To enable even distribution of this small amount of inoculum over the plot it was mixed thoroughly with 1500 ml of ground perlite (Cyclotec 1093 mill, to <1 mm particle size) moistened (with approx. 150 ml water) and then evenly applied to each appropriate plot. Inocula of Conio for the first, second and third crop, respectively consisted of 3.2×10^5, 1.7×10^5 and 4.5×10^5 cfu cm^{-3} reduced rate ground maizemeal-perlite which gave 1.3×10^8, 0.7×10^8 and 1.9×10^8 cfu m^{-2} soil, respectively and was recovered at 2.6×10^4, 0.1×10^4 and 0.6×10^2 cfu cm^{-3} soil after the first, second and third applications, respectively.

For spore suspension soil treatments, 3 litre of each spore suspension was evenly applied to appropriate plots using a watering can fitted with a fine rose, taking care to avoid splashing onto adjacent plots, left overnight, and then raked into the soil surface to a depth of about 3 cm. The spore suspension contained for the first crop $1.0–2.4 \times 10^5$ cfu ml^{-1}, the second crop $1.3–2.6 \times 10^5$ cfu ml^{-1} and for the third crop $0.7–1.1 \times 10^5$ cfu ml^{-1}. This gave $0.9–2.0 \times 10^8$ cfu m^{-2} soil for the first crop, $1.0–2.1 \times 10^8$ cfu m^{-2} soil for the second crop and $0.6–0.9 \times 10^8$ cfu m^{-2} soil, respectively for the third crop. This gave recovery of $0.6–5 \times 10^2$ cfu cm^{-3} soil for the first crop, $5.1–0.1 \times 10^3$ cfu cm^{-3} soil for the second crop and $8.0–0.2 \times 10^2$ cfu cm^{-3} soil for the third crop. Lettuce plants (cv. Titania for crop 1 and Rachel for crops 2 and 3) raised in peat blocks (Budge et al., 1995) were then immediately planted at $20 \times 20\,cm^2$ spacing in each plot at 7×13 plants per plot. Control plots receiving fungicidal sprays were treated with Rovral WP (0.5 g l^{-1} of wettable powder containing 50% w/w iprodione; May & Baker Ltd., Dagenham, UK) following a standard UK schedule. Glasshouse temperature was maintained at 12 °C day temperature with vents opening at 14 °C and with a 6 °C minimum night temperature. Insecticides were applied as necessary to control aphids.

Survival of antagonists in soil

At intervals throughout this trial, soil was taken from the top 3 cm in each plot, and survival of *C. minitans* assessed by soil dilution plating on PDA containing Triton X-100 (2 ml l^{-1}) and chlortetracycline (20 mg l^{-1}) (Whipps et al., 1989). *Coniothyrium minitans* isolates Conio, IVT1 and Contans® could be distinguished on dilution plates by colony morphology.

Assessment of disease, sclerotial numbers, viability and infection with Coniothyrium minitans

Each crop was harvested and assessed for the number of diseased plants, leaving diseased material on plots. Plants showing any signs of *Sclerotinia* infection were classed as diseased. Plants with severe *Botrytis* infection were excluded from assessments. Subsequently, the number of sclerotia on the soil surface was counted in five randomly positioned quadrats (500 cm^2 each) in each plot. A plant pot label was used to mark where each quadrat landed to avoid re-counting the same area. Ten sclerotia were collected from the first, third and fifth quadrats after the first and second crops, and twenty sclerotia after the third crop. If insufficient sclerotia were present in a quadrat, the nearest were chosen. The sclerotia were surface sterilised, bisected and placed on 15-mm dia. PDA discs containing chlortetracycline (20 mg l^{-1}) (Williams et al., 1998a). The viability and infection of sclerotia by *C. minitans* were assessed after 10–14 days incubation at 18 °C.

Glasshouse pot bioassay

Glasshouse pot bioassays based on those devised by McQuilken and Whipps (1995), were set up to assess the effect of soil-incorporated maizemeal-perlite and spore suspension inocula of IVT1, Conio and Contans® WG on apothecial production and *C. minitans* infection of sclerotia buried in treated soil.

Glasshouse soil was passed through a 5-mm sieve and adjusted to approximately 12% moisture content (−0.1 MPa). Maizemeal-perlite inocula of Conio were mixed thoroughly with batches of soil (3% v/v) by passing the soil through a 10-mm sieve three times prior to filling square plant pots (9 × 9 cm; Plantpak Ltd., Essex, UK). For the ground maizemeal-perlite inocula the amount required to give the same inoculation rate as the normal maizemeal-perlite was calculated (2.2% v/v) and mixed thoroughly with batches of soil before filling the pots. The amount of ground maizemeal-perlite inocula (reduced rate ground maizemeal-perlite inocula) required to give the same inoculation rate as for spore suspension was calculated (0.01% v/v). To enable even mixing of this small amount of inoculum it was mixed thoroughly with moistened (approx. 10% v/v) ground perlite (Cyclotec 1093 mill, to <1 mm particle size) to give the same volume as applied with the ground inoculum at full rate and then mixed thoroughly with batches of soil. Similarly, spore suspensions of either strain were applied to batches of soil and thoroughly mixed prior to filling pots. Soil treated with maizemeal-perlite inocula or spore suspensions and reduced rate maizemeal perlite inocula received approximately 10^7 or 10^3 cfu cm^{-3} soil, respectively. These concentrations were chosen to reflect application rates in the glasshouse. Terylene net bags (5 × 5 cm^2, mesh <2 mm) containing twenty sclerotia prepared as described earlier were then buried in each pot of inoculated soil approximately 1 cm below the surface. Pots were buried in a plot (0.85 × 2 m^2) in the glasshouse chamber, such that the soil surface in the pots was level with the soil surface in the glasshouse. Control treatments consisted of pots containing bags of sclerotia buried in uninoculated soil. Ten replicates of the eight treatments were arranged following an extended 8 by 8 Latin square. Each of the ten rows of eight pots contained a complete replicate set of treatments, with each of the eight columns of ten pots containing each treatment at least once. The additional pair of treatments in each column was arranged so that the same pair did not appear in more than one column. Conditions in the glasshouse were as described for the glasshouse lettuce trial, except that pots were not subjected to overhead irrigation. Instead, they were watered at weekly intervals using a watering can fitted with a fine rose, to maintain soil moisture, taking care to avoid splashing soil between pots. Three pot bioassays were set up (27th August 1998, 29th October 1998 and 24th March 1999), corresponding to the inoculum used in the first, second and third glasshouse lettuce crop.

Pots were examined for apothecia at weekly intervals for approximately six months. When no more apothecia were recorded, the bags of sclerotia were then removed from each pot, washed under a flow of tap water, and the number of sclerotia remaining counted. Recovered sclerotia were surface sterilised and assessed for infection with *C. minitans* as previously described.

Statistical analysis

For comparison between substrates, colony forming unit counts and conidia counts were $\log_{10}$ transformed before analysis using ANOVA (analysis of variance) assuming a completely randomised design.

Colony forming unit (cfu) survival data in glasshouse soil and pot bioassays were $\log_{10}$ transformed, following the addition of 0.375 to cope with zero counts, before analysis. For the glasshouse trial, lettuce quality data were expressed as percentage of the number

harvested, with these percentages arcsin transformed prior to analysis. Sclerotia numbers were square root transformed before analysis. Sclerotial viability and infection data were expressed as percentages of the number tested, with these percentages arcsin transformed prior to analysis. For the pot bioassays, apothecial numbers were square root transformed prior to analysis. Percentage sclerotial recovery, viability and infection data were arcsin transformed before analysis.

For both the glasshouse and pot bioassay trials, a Restricted Maximum Likelihood (REML) analysis was used to allow appropriate adjustment to be made for the structure of the designs (Thompson and Welham, 2000). This analysis allows account to be taken of the differences between the rows and columns of the design, despite the unbalanced nature of the treatment allocations, calculating treatment means adjusted for these differences. The analysis also allowed the assessment of the main effects of both strain and application methods, and comparison between controls and biological control treatments.

As the number of recovered sclerotia for the pot trial varied between treatments, the sclerotial viability and infection data were also analysed as counts within the Generalised Linear Model (GLM) framework, assuming a binomial error distribution and logit link function. This analysis approach allows a correct adjustment for the variation in the number tested (recovered) and also accounts for the variation in the number responding (viable or infected). This approach, however, cannot take proper account of the lack of balance imposed by the row and column structure of the design. As this analysis gave similar results to the REML analysis, only the results of the REML analysis are presented.

All comparisons noted are made at the 5% significance level.

Results

Conidial production and germination

Large numbers of conidia were produced (1.6×10^7 conidia g^{-1} substrate) on both maizemeal-perlite and ground maizemeal-perlite after 28 days, resulting in colony forming unit counts of $1.3–1.6 \times 10^{10}$ cfu g^{-1} substrate. Conidia from both solid-substrate media were highly germinable (94–100%) after 36 h incubation at 20 °C.

Glasshouse trial

Quadrat counts of sclerotia on the soil surface in each plot after harvest of the infection crop showed that the mean number of sclerotia per quadrat (500 cm^2) ranged from 0 to 1.0, and were generally evenly distributed throughout the glasshouse chamber.

Survival of Coniothyrium minitans *in soil*

Coniothyrium minitans Conio was detected at approximately $10^5–10^6$ cfu cm^{-3} soil in plots treated with maizemeal-perlite inoculum throughout the trial (Figure 1). Due to the application rate being close to the limit of detection it was not always possible to accurately enumerate *C. minitans* in plots treated with spore suspensions or reduced rate maizemeal-perlite, but Conio, IVT1 and Contans were consistently detected between approximately 10^1 and 10^4 cfu cm^{-3} soil.

Disease assessment

Disease levels in the untreated control were 3%, 29% and 57% in the first, second and third crops, respectively (Table 1). No significant difference in the percentage disease was recorded between any treatments in the first crop (Table 1). In the second crop, the fungicide treatment significantly reduced Sclerotinia disease compared with all other treatments. Disease was also significantly reduced by Conio maizemeal-perlite soil incorporation compared with the untreated control, but there was no significant difference in disease levels with the other *C. minitans* treatments. In the third crop, both the fungicide and the Conio maizemeal-perlite soil incorporation significantly reduced the percentage of diseased lettuce compared with the untreated control and there was no difference between these. The fungicide also significantly reduced the percentage disease recorded compared with all four *C. minitans* spore suspension treatments (Conio maizemeal-perlite derived spore suspension, IVT1 oats and maizemeal-perlite derived spore suspension and Contans® WG spore suspension) and Conio reduced rate maizemeal-perlite soil incorporation treatments.

There was no significant effect on percentage disease between the untreated control, *C. minitans* spore suspension treatments (Conio, IVT1 and Contans® WG) and Conio reduced rate maizemeal-perlite soil incorporation in any of the three crops.

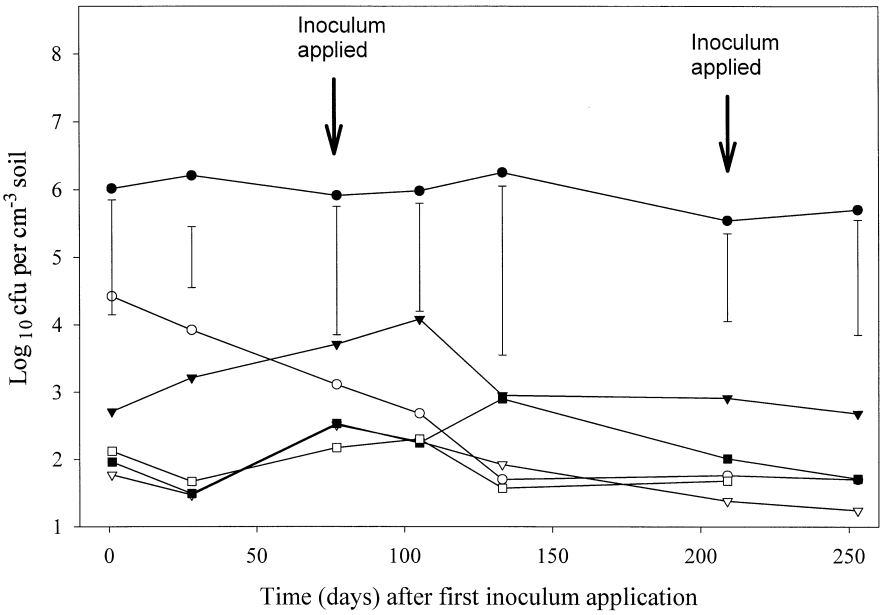

Figure 1. Survival ($\log_{10}$ cfu cm^{-3} soil) of *Coniothyrium minitans* in glasshouse soil after application of maizemeal-perlite or spore suspension inoculum at 0, 11 and 30 weeks. ●, Conio maizemeal-perlite full; ○, Conio maizemeal-perlite reduced; △, Conio spore suspension derived from maizemeal-perlite; ▲, IVT1 spore suspension derived from maizemeal-perlite; ■, IVT1 spore suspension derived from oat; □, Contans® WG spore suspension. Bars representing LSD (5% significance level, 12 d.f.) at each time interval based on analysis of variance of $\log_{10}$ of transformed data.

Table 1. Effect of soil incorporation of spore suspensions (spore) and maizemeal-perlite (mp) inocula of *C. minitans* Conio, IVT1 and Contans® WG on the percentage of *Sclerotinia*-diseased lettuce plants, the number of sclerotia recovered from the soil surface and the viability of the sclerotia after three sequential lettuce crops. Values in parentheses are means after arcsin transformation of data for percentage disease and percentage viability and square root transformations for number of sclerotia

Treatment	Extent of disease (%)			Mean no. sclerotia 2500 cm^{-2}			Viability of sclerotia (%)	
	1st crop	2nd crop	3rd crop	1st crop	2nd crop	3rd crop	2nd crop	3rd crop
Untreated control	3.1 (10.2)	28.5 (32.3)	57.1 (49.1)	1.0 (1.2)	34 (5.9)	260 (16.2)	82 (65.2)	94.8 (76.4)
Fungicide	2.2 (8.6)	0.5 (4.1)	15.7 (23.3)	1.0 (1.2)	0 (0.8)	19 (4.4)	NA[1]	95.8 (78.1)
Conio mp full	0.8 (5.1)	15.1 (22.9)	24.8 (29.9)	0 (0.6)	12 (3.5)	31 (5.7)	48 (44.0)	72.1 (58.1)
Conio ground mp reduced	0 (0.2)	19.7 (26.4)	43.9 (41.5)	0 (0.6)	19 (4.4)	193 (13.9)	69 (56.2)	91.4 (72.9)
Conio spore (mp)	0.3 (3.2)	21.6 (27.7)	37.8 (37.9)	0.8 (1.1)	22 (4.8)	116 (10.8)	81 (64.1)	96.6 (79.3)
IVT1 spore (mp)	1.6 (7.3)	22.8 (28.5)	46.5 (43.0)	0.5 (0.9)	12 (3.6)	101 (10.0)	62 (52.0)	96.4 (79.1)
IVT1 spore (oats)	0.5 (4.0)	20.6 (27.0)	46.5 (43.0)	0.7 (1.1)	33 (5.8)	256 (16.0)	67 (54.8)	94.2 (76.0)
Contans® WG spore	0.5 (4.0)	20.9 (27.2)	51.0 (45.6)	0.3 (0.8)	18 (4.3)	176 (13.3)	71 (57.4)	97.8 (81.4)
LSD[2] (5%, 7 d.f.)	(10.24)	(7.85)	(13.90)	(1.37)	(2.02)	(3.87)	(16.60)[3]	(16.27)

[1]Not applicable as no sclerotia present. [2]Significant differences between any treatment mean are calculated from the least significant difference (LSD), where LSD = $t_v \times$ SED, and SED = Standard error of the difference between the means derived from REML (Restricted Maximum Likelihood) analysis and t = critical value ($P = 0.05$) of Student's t distribution for v degrees of freedom (d.f.). LSD based on the maximum SED from the REML analysis. [3]LSD based on 6 d.f.

Sclerotial numbers, infection and viability

The mean number of sclerotia recovered from the soil surface after each of the three crops are shown in Table 1. The number of sclerotia recovered after the first crop was very low (0–1 sclerotium), with no significant differences among treatments. After the second crop, the fungicide control significantly reduced the number of sclerotia recovered (0 sclerotia)

compared with the untreated control (34 sclerotia) and any one of the *C. minitans* treatments (12–33 sclerotia). Significantly fewer sclerotia were also present in plots treated with Conio maizemeal-perlite inoculum (12 sclerotia) and IVT1 spore suspension produced from maizemeal-perlite (12 sclerotia) compared with the untreated control plots and those treated with IVT1 spores suspension derived from oats (33 sclerotia). Plots treated with Conio spore suspension derived from maizemeal-perlite, Contans® WG spore suspension or Conio reduced rate maizemeal-perlite (22, 18 and 19 sclerotia, respectively) did not differ significantly in the number of sclerotia recovered compared with Conio maizemeal-perlite inoculum and IVT1 spore suspension produced from maizemeal-perlite, nor with the untreated control and plots treated with IVT1 spores suspension derived from oats.

For the third crop, both the fungicide (19 sclerotia) and the Conio maizemeal-perlite inoculum (31 sclerotia) treated plots significantly reduced the number of sclerotia recovered on the soil surface compared with the untreated control (260 sclerotia) and all other *C. minitans* treatments (101–256 sclerotia). Plots treated with Conio and IVT1 spore suspensions derived from maizemeal-perlite also significantly reduced the number of sclerotia recovered (116 and 101 sclerotia, respectively) compared with the untreated control plots and those treated with IVT1 spores suspension derived from oats (256 sclerotia), but not compared with Contans® WG spore suspension (176 sclerotia) or Conio reduced rate maizemeal-perlite (193 sclerotia) treated plots.

The viability of sclerotia recovered from the soil surface after three sequential lettuce crops is shown in Table 1. The number of sclerotia recovered from the soil surface after the first crop was low and therefore no sclerotial samples were taken to assess viability and infection with *C. minitans*. After the second crop, Conio maizemeal-perlite soil incorporation treatment significantly decreased the percentage viability of sclerotia (48%) compared with the untreated control (82%) and plots treated with Conio spore suspension (81%), but not compared with any other treatment. No data were available for the fungicide control plot as no sclerotia were recovered. There was no significant difference in the viability of sclerotia recovered from plots treated with Conio spore suspension, Conio reduced rate ground maizemeal-perlite, IVT1 spore suspension derived from maizemeal-perlite, IVT1 spore suspension derived from oats, Contans® WG spore suspension and untreated control plots.

After the third crop, only Conio maizemeal-perlite soil incorporation treatment significantly decreased percentage viability of sclerotia compared with all treatments apart from Conio reduced rate maizemeal-perlite.

The data for the percentage sclerotia recovered after each of the second and third crops infected with *C. minitans* are shown in Table 2. No data is given for the fungicide treated plots for the second crop as no sclerotia were recovered from any of the plots. After both the second and third crop, the percentage of sclerotia infected with *C. minitans* was significantly higher in plots treated with Conio maizemeal-perlite (83.0% and 41.4%, respectively) compared with the untreated control plots (27.4% and 3.9%, respectively).

For the second crop, apart from the IVT1 spore suspension derived from maizemeal-perlite, none of the spore suspension or the Conio reduced rate maizemeal-perlite treatments significantly increased percentage infection of sclerotia compared to the untreated control. Similarly for the third crop, none of the spore suspension or Conio ground maizemeal-perlite at reduced rate treatments significantly increased percentage infection of sclerotia compared to the untreated and fungicide controls.

No significant difference was observed, in the percentage of sclerotia infected with *C. minitans* in the untreated control and fungicide treatment after the third crop.

After both the second and third crop a significantly higher percentage of sclerotia recovered from plots treated with Conio maizemeal-perlite were infected with Conio than those recovered from Conio spore suspension treated plots. For the second but not the third crop only, Conio infection of sclerotia recovered from Conio maizemeal-perlite compared with Conio reduced rate maizemeal-perlite treated plots was also significantly higher.

For the second crop, sclerotia recovered from plots treated with IVT1, whether from spore suspension inocula derived from maizemeal-perlite or oats, were more likely to be infected with IVT1 than with Conio or Contans® WG (Table 2). Correspondingly, the same was true for Conio and Contans® WG treated plots for the second crop. After the third crop, however, the percentage of Conio infected sclerotia were only significantly higher in plots treated with Conio maizemeal-perlite and not with Conio as ground maizemeal-perlite at reduced rate or spore suspension (Table 2). IVT1 infection of sclerotia recovered from IVT1 spore suspension derived from oats treated plots

Table 2. Effect of soil incorporation of spore suspensions (spore) and maizemeal-perlite (mp) inocula of *C. minitans* Conio, IVT1 and Contans® WG on the percentage infection of sclerotia recovered from the soil surface after the second and third lettuce crops. Values in parentheses are means after arcsin transformation of percentage data

Treatment	Infection of sclerotia by Conio, IVT1 and Contans® WG (%)							
	Second crop				Third crop			
	Total	Conio	IVT1	Contans®	Total	Conio	IVT1	Contans®
Untreated control	27.4 (31.6)	19.4 (26.4)	5.9 (14.0)	0.3 (3.0)	3.9 (11.5)	3.3 (10.5)	0.2 (2.5)	0.2 (2.4)
Fungicide	NA[1]	—	—	—	3.0 (9.9)	2.6 (9.3)	0 (0)	0.2 (2.7)
Conio mp full	83.0 (62.6)	82.3 (65.1)	0.2 (2.8)	0 (0)	41.4 (40.0)	41.4 (40.0)	0.7 (4.9)	0.2 (2.4)
Conio ground mp reduced	52.2 (46.3)	48.8 (44.4)	4.7 (12.6)	1.5 (7.0)	16.8 (24.2)	16.8 (24.2)	0 (0)	0 (0)
Conio spore (mp)	42.3 (40.6)	42.1 (4.04)	1.5 (7.0)	0.1 (2.0)	4.9 (12.8)	4.9 (12.8)	0 (0)	0 (0)
IVTI spore (mp)	65.6 (54.1)	10.1 (18.5)	59.4 (50.4)	0.4 (3.5)	4.0 (11.6)	1.3 (6.7)	0.7 (4.9)	0 (0)
IVTI spore (oats)	44.7 (42.0)	12.8 (21.0)	35.2 (36.4)	0.1 (2.1)	10.7 (19.1)	4.8 (12.6)	7.1 (15.5)	0 (0)
Contans®WG spore	50.3 (45.2)	3.0 (10.0)	0 (0)	46.9 (43.2)	3.3 (10.5)	0.7 (4.9)	0.2 (2.5)	2.2 (8.5)
LSD (5%)[2]	(17.64)	(18.58)	(13.15)	(10.91)	(19.72)	(16.20)	(8.89)	(7.50)

[1]Not applicable as no sclerotia present. [2]See footnote of Table 1 for definition with LSD based on 6 d.f. for the second crop and 7 d.f. for the third crop.

was significantly higher compared with all other treatments. There was no significant difference in the percentage of infection of sclerotia with IVT1 between any of the other treatments. Similarly, Contans infection of sclerotia was significantly higher in plots treated with Contans® WG compared with all other treatments apart from the untreated control, fungicide and Conio maizemeal-perlite at full rate treated plots. Clearly, the *C. minitans* isolates did move between treatment plots and infection by one isolate did not exclude infection by another isolate.

Glasshouse pot bioassays

Similar results were obtained for all three pot bioassays therefore only the results for the first pot bioassay using the inoculum for the first glasshouse lettuce crop are presented.

The sclerotia were harvested after 32 weeks (10 March 1999). Conio maizemeal-perlite soil incorporation, Conio ground maizemeal-perlite at full and reduced rate soil incorporation significantly reduced the number of apothecia produced compared with the untreated control (Table 3). No significant difference in the apothecial production was seen between any of the spore suspension inocula treatments and the untreated control. All *C. minitans* isolate Conio treatments significantly reduced the percentage recovery of sclerotia (to between 43.2% and 69.1%) compared with the untreated control (90.8%) and the remaining treatments (87.9–92.3%). There was no significant difference in the percentage recovery of sclerotia in the Conio maizemeal-perlite (43.2%) and the ground maizemeal-perlite full rate (49.4%), with Conio maizemeal-perlite full rate significantly reducing sclerotial recovery compared with the Conio spore suspension (66.3%) and Conio ground maizemeal-perlite reduced rate (69.1%) treatments. All of the Conio maizemeal-perlite solid-substrate inocula (maizemeal-perlite and ground maizemeal-perlite at full and reduced rate) significantly reduced percentage viability of recovered sclerotia compared with the untreated control and spore suspension inocula. Within these, Conio ground maizemeal-perlite at full rate significantly reduced sclerotial viability compared with Conio maizemeal-perlite which in turn significantly reduced sclerotial viability compared with Conio ground maizemeal-perlite at reduced rate treatment. Viability of recovered sclerotia was not seen to differ significantly between any of the spore suspension treatments (94.9–99.9%).

Infection of sclerotia with *C. minitans* was significantly increased with Conio maizemeal-perlite (72.9%), ground maizemeal-perlite at full (72.1%) or

Table 3. Glasshouse pot bioassay: the effect of maizemeal-perlite (mp) and spore suspension (spore) inocula of *C. minitans* Conio, IVT1 and Contans® on the number of apothecia produced by sclerotia of *S. sclerotiorum* and the subsequent number of those sclerotia recovered and viable. Values in parentheses are means after square root transformation for apothecial numbers and arcsin transformation of percentage data

Treatment	Cumulative number of apothecia	Recovery (%)	Viability (%)	Infection (%)
Untreated control	19.9 (4.5)	90.8 (72.3)	98.3 (82.6)	4.0 (11.6)
Conio mp full	1.3 (1.3)	43.2 (41.1)	37.7 (37.9)	72.9 (58.7)
Conio ground mp full	0.3 (0.9)	49.4 (44.6)	0 (0)	72.1 (58.1)
Conio ground mp reduced	4.6 (2.2)	69.1 (56.2)	69.9 (56.7)	25.7 (30.4)
Conio spore (mp)	9.3 (3.1)	66.3 (54.5)	94.9 (77.0)	14.4 (22.3)
IVT1 spore (mp)	11.6 (3.8)	87.9 (69.7)	99.9 (88.5)	1.4 (6.7)
IVTI spore (oats)	13.9 (3.5)	92.3 (73.9)	97.4 (80.7)	4.7 (12.5)
Contans® WG spore	9.2 (3.1)	91.7 (73.3)	99.3 (85.4)	4.5 (12.2)
LSD (5%, 7 d.f.)[1]	(1.57)	(12.74)	(14.97)	(14.81)

[1]See footnote of Table 1 for definition.

reduced rate (25.7%) treatments compared with the untreated control (4.0%). Conio maizemeal-perlite and ground maizemeal-perlite at full rate also significantly increased *C. minitans* infection of recovered sclerotia compared with all other *C. minitans* treatments (1.4–25.7%).

For all spore suspension treatments between 2.0–7.9 $\times$ 10^3 cfu cm^{-3} soil was applied, for Conio ground maizemeal-perlite at reduced application rate 2.0 $\times$ 10^4 cfu cm^{-3} soil was applied and both Conio maizemeal-perlite and ground maizemeal-perlite at full rates between 5.0–7.9 $\times$ 10^6 cfu cm^{-3} soil was applied. In all cases *C. minitans* was recovered from soil immediately after application, with spore suspension inocula being recovered at 4.0–12.5 $\times$ 10^3 cfu cm^{-3} soil, Conio ground maizemeal-perlite reduced rate at 3.9 $\times$ 10^4 cfu cm^{-3} soil and both Conio maizemeal-perlite and ground maizemeal-perlite at full rates at 3.2–4.0 $\times$ 10^6 cfu cm^{-3} soil.

Discussion

Sclerotinia disease of lettuce in the glasshouse trial was only reduced by the maizemeal-perlite at full application rate treatment and not with the spore suspension or the maizemeal-perlite ground at reduced rate treatments. Similarly, only the maizemeal-perlite at full application rate consistently decreased the number and viability of sclerotia recovered post harvest and increased infection of sclerotia compared with the untreated control. This result could reflect propagule levels, as maizemeal-perlite inoculum was applied at a much higher rate (0.8–1.1 $\times$ 10^{11} cfu m^{-2}) compared with the spore suspension (0.6–2.0 $\times$ 10^8 cfu m^{-2}) or the reduced rate ground maizemeal-perlite (0.7–1.9 $\times$ 10^8 cfu m^{-2}).

Coniothyrium minitans infection of sclerotia recovered from maizemeal-perlite plots was also high throughout the trial, which resulted in a reduction in sclerotial viability in the second and third crop. Again, the high inoculum concentration present in the maizemeal-perlite treatment is likely to result in greater contact of the mycoparasite with the sclerotia which is necessary for sclerotial infection (Whipps and Budge, 1990). Percentage viability was greater and infection of sclerotia with *C. minitans* lower in the third crop compared with the second crop in all cases. The duration of the second crop, however, was longer (16 weeks) compared with the third crop (10 weeks) and this additional period might allow *C. minitans* to infect and reduce sclerotial viability more effectively. Spore suspension and reduced maizemeal-perlite treatments were inconsistent in their effects.

In previous glasshouse lettuce trials, where a single *C. minitans* was applied before the first crop only, consistent control of *S. sclerotiorum* disease was only achieved when disease incidence was below 50%, however, as disease levels increased control was quickly lost (Lynch and Ebben, 1986; Whipps et al., 1989; Budge and Whipps, 1991; Budge et al., 1995). In the present study, disease control was achieved at 57% disease levels when *C. minitans* maizemeal-perlite inocula at full rate was applied prior to each of the three lettuce crops. Budge and Whipps (2001) suggested that repeated application of *C. minitans* prior to each crop rather than a single application to the first crop improves disease control as new sclerotia on the

soil surface at the start of each crop will be exposed to this repeated *C. minitans* application.

Coniothyrium minitans survived in the soil throughout the glasshouse trial and pot bioassays and previous studies have shown *C. minitans* to survive in soil for much longer periods than recorded in this trial (at least 3 years) (McQuilken et al., 1995). This ability to survive in soil may therefore be important for long term Sclerotinia-disease control. In addition, with all treatments, the recovery of *C. minitans* was consistent with the amount applied, with little decrease in numbers during each crop or pot bioassay. *Coniothyrium minitans* infected sclerotia were also recovered from both the control plots in the glasshouse trial and the control pots in the glasshouse bioassay. Water splash from overhead irrigation, and soil mesofauna have been reported to spread *C. minitans* from the site of application to other plots in glasshouse trials (Williams et al., 1998a–c) and may have occurred in these experiments. Such dispersal mechanisms may again be important for long term control of *S. sclerotiorum* in glasshouse crops. All *C. minitans* isolates were observed in other treated and untreated plots and more than one *C. minitans* isolate could be recovered from an individual sclerotium. This indicates that infection by one *C. minitans* isolate did not exclude infection by another, but the isolate applied to plots was seen to infect the greatest number of sclerotia recovered from those plots. Spore sprays containing a mixture of *C. minitans* isolates have been used by other workers (Evenhuis et al., 1995; Gerlagh et al., 1999) to control Sclerotinia disease, though no information on the relative infection of the sclerotia by the different isolates was given. This is the first report of dual infection of sclerotia by different *C. minitans* isolates.

In the pot bioassay, full rate maizemeal-perlite reduced the number of apothecia produced, the percentage of sclerotia recovered and the percentage viability of the sclerotia more than the reduced rate. This reflects the differences in the inoculum concentration applied, 10^{11} cfu m^{-2} (3.3×10^{6} cfu cm^{-3}) for full rate maizemeal-perlite treatments and 10^{8} cfu m^{-2} (3.3×10^{3} cfu cm^{-3}) for the spore suspension or reduced rate maizemeal-perlite treatments and is consistent with the results of the greenhouse trial. Additionally, although the maizemeal-perlite ground reduced rate inoculum of *C. minitans* was applied at a similar rate to the spore suspension inocula, only the maizemeal-perlite ground reduced rate inoculum significantly reduced the number of apothecia produced and viability of sclerotia recovered and increased sclerotial infection compared with the untreated control. This could be due to *C. minitans* surviving better as a solid substrate incorporation compared to a spore suspension inoculum. In addition, the maizemeal in the medium may attract soil mesofauna, thus facilitating infection of sclerotia (Williams et al., 1998a).

Ground maizemeal-perlite supported growth and sporulation of *C. minitans* as well as the normal maizemeal-perlite. Further, in the pot bioassay there was no difference in the ability of the two full rate maizemeal-perlite treatments to reduce apothecial numbers, reduce percentage sclerotial recovery and increase infection of sclerotia. These two full rate maizemeal-perlite inocula were highly effective at infecting sclerotia and reducing apothecial production and the results are consistent with the findings of McQuilken and Whipps (1995).

In conclusion, for both the glasshouse lettuce trials and pot bioassay, inoculum level appears to be a key factor in both control of Sclerotinia disease and suppression of apothecial production by sclerotia, with an indication that the optimum rate for disease control is between 10^{8} (10^{1}–10^{4} cfu cm^{-3} soil) and 10^{12} (10^{6} cfu cm^{-3} soil). For control of Sclerotinia disease in glasshouse lettuce, the recommended rate for the commercial *C. minitans* product Contans® WG is $2–4 \times 10^{8}$ cfu m^{-2} but this treatment was not successful in reducing disease in this study. However, a period of at least 8 weeks between inoculum application and planting of crop to enable the *C. minitans* inoculum to infect sclerotia and reduce sclerotial viability is recommended for this product. Further work should investigate optimising the timing and *C. minitans* application rate in relation to crop planting, in order to maximise the efficacy of the biocontrol treatment with a minimum period between crops. This is important for all year round lettuce but may be less so for field crops such as oilseed rape or sunflowers when inoculum application can be made in the autumn prior to planting.

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References

Boland GJ and Hall R (1994) Index of plant hosts of *Sclerotinia sclerotiorum*. Canadian Journal of Plant Pathology 16: 93–108

Budge SP and Whipps JM (1991) Glasshouse trials of *Coniothyrium minitans* and *Trichoderma* species for the biological control of *Sclerotinia sclerotiorum* in celery and lettuce. Plant Pathology 40: 59–66

Budge SP, McQuilken MP, Fenlon JS and Whipps JM (1995) Use of *Coniothyrium minitans* and *Gliocladium virens* for biological control of *Sclerotinia sclerotiorum* in glasshouse lettuce. Biological Control 5: 513–522

Budge SP and Whipps JM (2001) Potential for integrated control of *Sclerotinia sclerotiorum* in glasshouse lettuce using *Coniothyrium minitans* and reduced fungicide application. Phytopathology 91: 221–227

Challen MP and Elliott TJ (1986) Polypropylene straw ampoules for the storage of microorganisms in liquid culture. Journal of Microbiological methods 5: 11–23

Coley-Smith JR and Cooke RC (1971) Survival and germination of fungal sclerotia. Annual Review of Phytopathology 9: 65–92

Durand A, Renaud R, Maratray J, Almanza S and Pelletier A (1994) Reactor for sterile solid-state fermentation methods. Patent No. WO 94/18306

Evenhuis A, Verdam B, Gerlagh M and Goossen-van de Geijn HM (1995) Studies on major diseases of caraway (*Carum carvi*) in the Netherlands. Industrial Crops and Products 4: 53–61

Gerlagh M, Goossen-van de Geijn HM, Fokkema NJ and Vereijken PFG (1999) Long term biosanitation by application of *Coniothyrium minitans* on *Sclerotinia sclerotiorum* infected crops. Phytopathology 89: 141–147

Huang HC (1980) Control of sclerotinia wilt of sunflower by hyperparasites. Canadian Journal of Plant Pathology 2: 26–32

Huang HC, Bremer E. Hynes RK and Erickson RS (2000) Foliar application of fungal biocontrol agents for the control of white mold in dry bean caused by *Sclerotinia sclerotiorum*. Biological Control 18: 270–276

Jones EE and Stewart A (2000) Selection of mycoparasites of sclerotia of *Sclerotinia sclerotiorum* isolated from New Zealand soils. New Zealand Crop and Horticulture Journal 28: 105–114

Lynch JM and Ebben MH (1986) The use of micro-organisms to control plant disease. Journal of Applied Bacteriology Symposium Supplement 1986, 115S–126S

McQuilken MP and Whipps JM (1995) Production, survival and evaluation of solid-substrate inocula of *Coniothyrium minitans* against *Sclerotinia sclerotiorum*. European Journal of Plant Pathology 101: 101–110

McQuilken MP, Mitchell SJ, Budge SP, Whipps JM, Fenlon JS and Archer SA (1995) Effect of *Coniothyrium minitans* on sclerotial survival and apothecial production of *Sclerotinia sclerotiorum* in field-grown oilseed rape. Plant Pathology 44: 883–896

McLaren DL, Huang HC, Kozub GC and Rimmer SR (1994) Biological control of sclerotinia wilt of sunflower with *Talaromyces flavus* and *Coniothyrium minitans*. Plant Disease 78: 231–235

Merriman PR (1976) Survival of sclerotia of *Sclerotinia sclerotiorum* in soil. Soil biology and Biochemistry 8: 385–389

Mylchreest SJ and Wheeler BEJ (1987) A method for inducing apothecia from sclerotia of *Sclerotinia sclerotiorum*. Plant Pathology 36: 16–20

Thompson R and Welham SJ (2000) REML analysis of mixed models. In: Payne RW (ed) The Guide to GenStat, Part 2; Statistics (pp 413–503). VSN International Ltd., Oxford, UK

Tribe HT (1957) On the parasitism of *Sclerotinia trifoliorum* by *Coniothyrium minitans.* Transactions of the British Mycological Society 40: 489–499

Trutmann P, Keane PJ and Merriman PR (1980) Reduction of sclerotial inoculum of *Sclerotinia sclerotiorum* with *Coniothyrium minitans*. Soil Biology and Biochemistry 12: 461–465

Verdam B, Gerlagh M and van de Geijn HM (1993) Biological control of *Sclerotinia sclerotiorum* in caraway (*Carum carvi*). Mededelingen van de Faculteit Landbouwwetenschappen Universiteit Gent 58: 1343–1347

Whipps JM and Budge SP (1990) Screening for sclerotial mycoparasites of *Sclerotinia sclerotiorum*. Mycological Research 94: 607–612

Whipps JM, Budge SP and Ebben MH (1989) Effect of *Coniothyrium minitans* and *Trichoderma harzianum* on *Sclerotinia* disease of celery and lettuce in the glasshouse at a range of humidities. In: Cavalloro R and Pelerents C (eds) Integrated Pest Management in Protected Vegetable Crops, Proceedings of the CEC IOBC Group Meeting Cabrils 27–29 May, 1987 (pp 233–243) A.A. Balkema, Rotterdam, the Netherlands

Whipps JM and Gerlagh M (1992) Biology of *Coniothyrium minitans* and its potential for use in disease biocontrol. Mycological Research 96: 897–907

Whipps JM, McQuilken MP and Budge SP (1993) Use of fungal antagonists for biocontrol of damping-off and *Sclerotinia* diseases. Pesticide Science 37: 309–313

Williams RH, Whipps JM and Cooke RC (1998a) The role of soil mesofauna in dispersal of *Coniothyrium minitans*: transmission to sclerotia of *Sclerotinia sclerotiorum*. Soil Biology and Biochemistry 30: 1929–1935

Williams RH, Whipps JM and Cooke RC (1998b) The role of soil mesofauna in dispersal of *Coniothyrium minitans*: mechanism of transmission. Soil Biology and Biochemistry 30: 1937–1940

Williams RH, Whipps JM and Cooke RC (1998c) Water splash dispersal of *Coniothyrium minitans* in the glasshouse. Annals of Applied Biology 132: 77–90